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ROYAL COMMISSION ON TUBERCULOSIS (HUMAN AND ANIMAL).

SECOND INTERIM REPORT

OF THE

ROYAL COMMISSION

APPOINTED TO INQUIRE INTO THE RELATIONS OF

HUMAN AND ANIMAL TUBERCULOSIS

PART II. APPENDIX.

VOLUME III.

ADDITIONAL INVESTIGATIONS OF BOVINE & HUMAN VIRUSES

BY

DR. L. COBBETT, DR. A. STANLEY GRIFFITH, & DR. F. GRIFFITH.

Presented to both Houses of Parliament by Command of His Majesty.

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PREFACE.

The details and results of the experiments made to determine the relative virulence and pathogenic effects of tuberculous viruses of Bovine and Human origin on various species of animals have been published in the first and second volumes of the Appendix to the Second Interim Report of the Commission.

Whilst those experiments were in progress others, undertaken to throw light on collateral questions, were undertaken at Stansted, and the results of these additional investigations are collected together in this volume.

The results of ten separate investigations are included. Each of these was designed by, and carried out under the direct supervision of, the Commission, but the actual reports have been written by the investigators whose names are attached to them. In their own report (Part I.) the Commission expressed the opinions they themselves had founded on the results of the whole of the experimental work carried out at Stansted, and these general opinions were in every instance in complete accord with those of the resident investigators. In the individual reports contained in this volume it has been considered right to allow each of the investigators to set forth not only the definite facts arising out of the experimental work, but also his own views based on the work done by himself. (Report, Part I., page 42.)

E. J. S.

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THE CULTURAL CHARACTERS OF THE BOVINE TUBERCLE BACILLUS.

Dr. A. STANLEY GRIFFITH and Dr. F. GRIFFITH.

THE CULTURAL CHARACTERS OF THE BOVINE TUBERCLE BACILLUS.

This report gives the results of the investigation as to the cultural characters of the bovine tubercle bacillus.

The investigation has been carried out with two chief objects in view :

1. To find the most favourable media for the growth of the tubercle bacillus.
2. To contrast the cultural characters of different strains of bovine tubercle bacilli.

The cultures investigated were derived from bovine animals naturally infected with tuberculosis, and were isolated, in the majority of cases, direct from the original material.

300 strains of tubercle bacilli have been isolated, representing 30 viruses.*

Failures to get the bacillus to grow have been very few and occurred in the early part only, in the cases of a few guinea-pigs where either unsuitable media were used or an insufficient number of tubes sown.

The following is a list of the media used :—

Serum media. Serum has been obtained from 12 species of animals, namely, the ox (cow and calf), horse, pig, goat, sheep, baboon, monkey, lemur, dog, cat, rabbit, and guinea-pig.

Glycerin serum.

Serum agar, with and without glycerin.

Egg : the yolk and white have been used separately and combined.

Glycerin agar.

Glycerinated potato.

Glycerin broth.

ditto. with 10 per cent. gelatine.

Asparagin liquid medium.

Mode of Preparation of Media.

Serum media : The serum media are prepared in the following way : Blood is collected in sterile flasks from animals, either by inserting a cannula in the external jugular vein during life, or in the common carotid artery after shooting, or anæsthetising ; the blood of rabbits and guinea-pigs is usually collected by allowing the blood to flow direct into a large sterile test-tube pressed against the side of the neck after exposing and severing the carotid and jugular vessels.

The blood is allowed to stand from one to three or four days, according to the speed with which the serum separates ; the serum is then decanted, either by means of a pipette or a syphon, and distributed into tubes, about 6 cc. being used for each. The tubes are put in an inclined position in a serum inspissator, and kept for about two hours at a temperature that varies in different cases between 75°C. and 80°C. In the inspissator is placed a bowl containing water. Each tube is then sealed with paraffin and stored.

By this method the different kinds of sera, at the time of inoculation, necessarily varied in age and moistness.

To make the media more strictly comparable, the serum after distribution into test tubes was at one period fractionally sterilised (*i.e.* kept at 60°C. for an hour on five consecutive days) and stored in a liquid state in an ice chest, each tube having a rubber cap placed over its aperture to prevent evaporation. When required for use a series of tubes were coagulated as above. In this way it was thought that variations in the physical characters of the serum, produced by slight differences in the temperature and duration of the coagulation, and in the amount of moisture, would be eliminated.

The method was abandoned because it did not appear to possess enough advantage over the old way of preparing the serum to make it worth the extra trouble ; there was the distinct disadvantage that the tubes were never ready for immediate sowing and, probably on account of the concentration of the serum, a greater tendency to the formation of a film of salts on the surface was exhibited.

Glycerin serum : 5% sterile glycerin is added to a measured quantity of serum before distribution into tubes ; the procedure is then the same as for serum.

Serum agar is prepared by adding 0·5 cc. serum to 5 cc. agar liquefied and cooled down to about 50°C. ; the two are well mixed and sloped.

Egg mixture : fresh eggs are broken into a sterile beaker and the white and yolk beaten up with a sterile glass rod ; sterile salt solution is then added in the proportion of 1 to 3 and the mixture is distributed into tubes ; the tubes are sloped in the serum inspissator and kept for 2 hours at 75°C. on two consecutive days.

The yolk and white, when used separately, are coagulated in the same way as the egg mixture ; no saline is added.

Yolk agar is made in the same way as serum agar.

Glycerinated potato : the potato wedges are first soaked in a 1 in 1000 sodium carbonate solution for 24 hours and then in a 5 % glycerin solution deeply tinted with litmus until the potato acquires a definite blue colour. The potato is afterwards put into test tubes containing at the bottom a little cotton wool soaked in 5 % glycerin solution and sterilized in the usual way. Growth is readily observed on a potato tinted in this manner.

Glycerin agar, and glycerin broth are prepared after the usual formulae.

The asparagin medium was prepared by dissolving asparagin (5 grms.), KH_2PO_4 (5 grms.), sodium chloride (5 grms.), magnesium citrate (2·5 grms.), potassium sulphates (2·5 grms.), in distilled water to 1000 cc. and filtering the solution after reducing the acidity to 20 per litre.

Source of Cultures.

In the case of the viruses the direct culture has usually been obtained from the thoracic glands and occasionally from the lung, mesenteric glands or perlsucht growths on pleura or diaphragm.

In experimental bovines the material for inoculating the tubes has been taken from the prescapular, mesenteric thoracic or supramammary lymphatic glands, the prescapular gland being most often used.

In pigs the direct cultures have been raised from the submaxillary and bronchial glands.

In the guinea-pig the sternal gland is the most convenient gland from which to make cultures, but when the sternal gland is not much enlarged, the liver or the spleen has been chosen on account of the softness of the tissues of these organs.

The lesions in a monkey, rabbit or mouse are well adapted for the production of cultures ; they are usually not only rich in bacilli, particularly in the latter animal, but also, being softened, easy to emulsify and spread evenly over the surface of the medium.

Method of Raising Primary Cultures.

Whatever organ or gland is selected it is first freed from all surrounding connective tissue, dried and then well seared with a Paquelin cautery. An incision is made with a sterile knife in the centre of the seared area, and a small piece of tissue is removed.

In the early part of the work a bit of the tissue was deposited on the surface of each medium, and was then picked up with a stout platinum wire and carried to the condensation water at the foot of the slope ; here it was squeezed and pressed against the sides of the tube until the water became definitely milky ; the emulsion thus found was allowed, by slightly inverting the tube, to flow over the whole surface and the broken up pieces of tissue were well rubbed over every part of the medium.

When caseous and softened lesions were being dealt with an even distribution of the material was readily secured, but when the tissue was composed of the firm, dense, caseo-necrotic material met with in acute tuberculosis in bovines a good distribution was not so readily effected.

In such cases various modifications were tried ; at first pieces of the gland were broken up in a sterile mortar and emulsified with normal saline, a loopful of the emulsion being smeared over the surface of each medium. Then, instead of in a mortar, the piece of tissue was broken up and emulsified in a short stout test tube by means of a glass rod.

---

* Virus is the term applied to the original material ; a culture derived from that virus, either directly or through an experimental animal, is called a "strain."

The last method, the one now exclusively used, has several advantages ; a small piece of tissue which in the first method could be used for one tube only, can by this means be used to inoculate a large series and there are fewer risks of air-borne contaminations than when emulsified in a mortar ; there is also the advantage that the emulsion so prepared can be examined microscopically before sowing the tubes, and if too rich in bacilli, *e.g.* for the production of discrete colonies, can be diluted to any required extent, or if too poor a larger loop can be used for sowing the tubes.

After sowing, the tubes are resealed with paraffin and placed in the incubator in a vertical position. At one time, to prevent undue evaporation the tubes were placed in a position inclined so as to allow the water of condensation to flow over the greater part of the surface of the medium.

The practice was discontinued for primary cultures, because it was found that this position enabled contaminating organisms which, in the vertical position might have remained localised to one spot, to overrun the whole surface, thus rendering it impossible to secure a pure culture.

ESTIMATION OF THE VALUE OF VARIOUS MEDIA FOR PRIMARY CULTURES.

Many observers have experienced difficulty in raising cultures of the bovine tubercle bacillus on ordinary media. This investigation has been carried out to find what media are most suitable for the growth of the bacillus in primary culture and incidentally to ascertain whether the serum of different species of animals has any specific value. For this purpose whenever a culture was desired a series composed of as large a variety of media as was available at the time was sown. The results are given in tabular form.

Medium.	Number of tubes sown.	Number of Positive Results.	Percentage.	Medium.	Number of tubes sown.	Number of Positive Results.	Percentage.
Cat Serum - -	70	56	80	Cat Serum Agar - -	8	5	62
Goat ,, - -	83	53	64	Goat ,, ,, - -	26	23	80
Baboon ,, - -	36	22	61	Baboon ,, ,, - -	21	9	43
Rabbit ,, - -	45	27	60	Rabbit ,, ,, - -	41	15	37
Guinea-pig Serum -	48	29	60	Guinea-pig Serum Agar	2	1	50
Pig ,, -	191	116	60	Pig ,, ,,	20	3	15
Dog ,, -	198	112	57	Dog ,, ,,	74	59	80
Sheep ,, -	53	30	57				
Rhesus ,, -	89	44	50				
Adult Bovine ,, -	229	90	43	Adult Bovine ,, ,,	59	46	78
Calf ,, -	81	24	30	Calf ,, ,,	23	8	35
Horse ,, -	43	7	16	Horse ,, ,,	16	0	0
				Rat ,, ,,	10	0	0
				Lemur ,, ,,	2	2	100
Egg mixture - -	498	459	92	Glycerin Agar - -	88	5	6
Egg yolk - - -	19	17	90	Glycerinated potato -	144	25	17
Egg white - -	16	8	50	Glycerinated Serum -	148	19	13
Egg yolk agar - -	50	30	60	Glycerin Agar + 5 % dog Serum - -	38	15	42
				Glycerin Agar + 5 % pig Serum - -	16	9	56
				Glycerin Agar + 10% Bovine Serum - -	12	10	83

The egg medium has been the most successful and can always be relied upon for the production of primary cultures. It has the additional advantages of being readily obtained and easily prepared. The yolk and the egg mixture are of about equal value and far superior to the white alone.

On the first three media the colonies are visible in from eight to twelve days and appear as transparent points. If very numerous they remain small and transparent, but when there are only a few they may grow to a considerable size becoming raised and creamy white, and either moist, or dry and warty.

The sera of different species of animals have been found, as Professor MacFadyean predicted, to vary less than the sera of animals of the same species. The sera of a large number of animals of each species have been used and at least one serum of each species has given a high percentage of successes. The serum from the cat has been found the most uniformly good, while that of the horse up to the present has been generally poor. Several poor sera in the case of the dog have considerably lowered the high percentage of successes obtained at first. From the calf only occasionally has a good serum been obtained ; the adult ox, on the other hand, has yielded a serum which has been invariably good, especially when of a golden yellow colour. In several instances serum containing a large number of blood corpuscles has given better results than the same serum without corpuscles.

The failure to produce growth in the case of some sera appears often to be due to the formation of a film over the surface. It was chiefly to avoid this disadvantage that serum agar was used.

Serum agar is a satisfactory and economical substitute for pure serum, and has the advantage of being perfectly transparent ; it has been found to be very little inferior to the egg medium for raising primary cultures.

On several occasions a serum which has been found poor when coagulated, has given excellent results as serum agar.

Glycerin serum and potato are not very useful for primary cultures and when growth occurs the bacillus is usually one which grows well on glycerinated media in subculture. On potato in one instance a most luxuriant primary growth was obtained, much superior to any subculture on this medium.

On glycerin agar in only a few instances has a primary culture been obtained.

SUBCULTURES.

The tubercle bacillus having once started to grow on artificial media grows readily when transplanted on almost every variety of serum media although early generations grow less abundantly than later. Growth takes place from the outset and usually attains its maximum at the end of three weeks' incubation. On the same variety of serum all the viruses produce the same type of growth. On bovine serum the growth is in the form of a dry, usually uniform grey coherent layer; on dog serum it is moist and more easily emulsified and exhibits no tendency to spread over the condensation water; on pig serum the growth is scaly and more abundant than on bovine serum.

On different varieties of serum the amount of growth varies, being most abundant on those which give the best results in primary culture. The egg medium is unsatisfactory for subcultures.

The bovine tubercle bacillus will not grow at a temperature of 42°C. or when placed under anaerobic conditions.

The bacillus retains its vitality in culture for a considerable time and by means of the egg medium a growth was obtained from a subculture 597 days old (the virulence of this culture for rabbits remained unaltered.)

COMPARISON OF THE CULTURAL CHARACTERS OF THE DIFFERENT VIRUSES.

In these comparisons the importance of employing the same technique throughout was recognised, and the following method adopted.

A small amount of culture, approximately equal in all cases, was taken and smeared evenly over the surface of each medium; the even distribution was effected by emulsifying the material on the surface of the medium with a drop of the condensation water. By the use of a small amount of culture, the differences between the various strains are brought out more clearly than when a large amount is used. The most uniform results are obtained when the inoculated material is taken from a vigorous subculture.

The media used for the comparisons of the growth characters have been serum and glycerin serum, glycerin agar, glycerinated potato, and glycerin broth.

On glycerin serum the viruses of bovine origin exhibit three types of growth.

In the first, no growth can be observed until the second or third week, when discrete colonies make their appearance. In some cases the colonies remain small and discrete, in others they continue to grow, and the amount of growth at the end of six weeks may equal or exceed that on the corresponding pure serum. In this first series, the distinguishing features are the initial delay on glycerin serum compared with the steady uniform increase on pure serum, and the absence of any considerable variation between the strains which exhibit this type of growth.

In the second, growth takes place from the outset and up to the end of the second week is similar in appearance to that on serum alone, and about equal in amount. The amount of growth finally produced at the end of the third or fourth week varies in different strains; with Viruses II., VII., XXIX., and XXX. growth is about equal to that on pure serum, with Virus XVI. rather less, and with Viruses VIII., XIV., XX., XXII., XXIII., and XXVII. rather more than on the corresponding serum.

In the third, growth during the first week is more abundant than on pure serum, and progressively increases, becoming, at the end of the fourth week, from two to four times as much as on serum. The characters of the growth also differ markedly on the two media; that on serum is grey and uniform with, in many cases, scattered raised colonies on the surface, while that on glycerin serum is whiter and moister with a more irregular and often wrinkled surface.

The bovine viruses which exhibit the first type of growth are Nos. I., III., IV., VI., XII., XIII., XV. XVII., XVIII., XIX., XXI., XXIV., and XXVI.

Those which exhibit the second type are II., VII., VIII., XIV., XVI., XX., XXII., XXIII., XXVII., XXIX., and XXX.

Those which exhibit the third type are V., IX., X., XI., XXV., and XXVIII., the latter growing rather less well than the others.

On pure serum the characters of the growth are the same for all viruses, and no differentiation on this medium can be made after they have been in cultivation some time. A uniform grey layer, often with raised white colonies on the surface, is formed at the end of the first week; this increases until the third week, when in the majority of cases growth ceases.

Viruses of Class I. do, however, grow in early generations less readily on serum than those of Class III., thus showing that the differences between the various viruses brought out by the use of glycerinated media are differences in saprophytic power and are in freshly isolated cultures capable of demonstration on pure serum.

On glycerin agar the results are in accordance with those on glycerin serum, and the same differentiation of the viruses can be made.

The first series of viruses grows with difficulty; in some cases no growth whatever occurs, in others discrete colonies make their appearance after several weeks, sometimes in the fourth, and often much later. As in the case of glycerin serum, there is the initial delay before the appearance of colonies, which when numerous may coalesce to form a thin continuous layer, and when fewer and discrete may attain a considerable size, becoming heaped up, warty, and slightly pigmented.

In the second series growth takes place from the outset, a thin grey layer being formed at the end of the first week, which increases slightly up to the end of the third week. Subsequently, in some cases, raised colonies develop on the surface.

In the third, growth is more abundant, and attains a moderate thickness at the end of the third week, showing in some cases a fine wrinkling on the undersurface but no pigmentation. In the case of Virus V., however, a definitely wrinkled and slightly pigmented layer was obtained.

On glycerinated potato growth is very variable, but in the main corresponds with that on glycerin agar. The viruses in the first and second series form after many weeks discrete colonies, which grow slowly becoming pearly-white and up to a pinhead in size. Occasionally larger, irregular, creamy nodules are obtained after several months. Those in the third series grow more readily, and produce in from two to three weeks a grey layer, varying in thickness, in which raised warty yellowish colonies may subsequently develop. The growth of viruses in all the series may be attended with the formation of pigment to some extent, varying from creamy white to a definite yellow.

On glycerin broth great difficulty was experienced in getting many of the viruses to grow when inoculated direct from serum. This has been especially the case with viruses in the first series, the inoculated material either not increasing or forming warty nodules, which might, after many weeks, send out over the surface a very thin grey membrane.

On the asparagin medium, growth was as a rule more readily obtained than on broth, but the pellicle formed was often incomplete and in all cases thin and uniform. As all the viruses produced more or less the same type of pellicle, little differentiation was exhibited on this medium.

Various devices have been adopted to enable the inoculated material to form a pellicle on the surface. In one the broth in six-ounce medicine bottles, contained a pledget of cotton wool which just projected above the surface of the fluid, and on to which the culture was inoculated. Growth took place on the wool in the form of white or creamy irregular nodules from which after a long time a delicate grey pellicle developed.

In another, 10 per cent. gelatine was added to the broth forming at 37°C. a liquid medium. This medium gives uniform results since, being solid at room temperature it can be inoculated from young serum cultures of equal ages; it has the additional advantage, that by allowing it to solidify and adding formalin the pellicles can be preserved as permanent specimens. The characters of the growth do not differ from those of glycerin broth cultures.

The method devised by Dr. Eastwood of combining solidified serum and broth has also been attended by constant results in producing surface growths characteristic of the different viruses.

Those viruses which grow with most difficulty on glycerinated media show a corresponding reluctance to grow on the surface of glycerin broth; the type of pellicle formed is delicate grey and almost transparent, and the growth is occasionally broken up into islands which incompletely cover the surface.

Viruses which grow more readily on glycerinated media generally grow more rapidly on broth, and produce a thicker translucent pellicle with opaque patches. In all cases, after the pellicle has begun to form, the surface of the broth is quickly covered, and there is little subsequent thickening, the growth often showing a tendency to break up, become moist and sink.

A consideration of the results on the differential media shows that bovine bacilli can be arranged in a more or less complete series beginning with a bacillus which grows badly on all the differential media and ending with one which grows relatively well. On this point we are in entire agreement with Dr. Eastwood in his classification. We have not, however, attempted to arrange the strains in a consecutive series; our experience with various culture media makes us hesitate to rely too much upon small points of difference.

Nevertheless, there is a marked contrast between the extreme types of growth on all media containing glycerin. For example, a comparison of the weight of growth produced on serum and glycerin serum, respectively, has been made with strains representing each series.

The method adopted was to sow in each case a number of tubes of serum and glycerin serum and incubate for three weeks. An equal number of tubes on which the best growths had occurred was then selected and the growth scraped off and weighed. In the case of Virus 20 the growth after being weighed directly was emulsified with saline and estimated by the volumetric method devised for culture inoculations. The weight as estimated by this method was found to agree closely with that obtained on the balance.

TABLE OF COMPARISONS OF WEIGHTS.

Virus.	Number of tubes on each medium.	Weight on Serum.	Weight on Glycerin Serum.	Ratio.
B. IV.	4	53	5	10 : 1
B. XX.	4	100	120	1 : 1·2
B. V.	6	90	300	1 : 3·3
B. IX.	4	35	142	1 : 4

In the above comparisons the same serum of excellent quality was employed. When only a moderate serum is used the differences are more marked, as in the following cases which were incubated for 4 weeks.

B. X. G.P. 1072	1	4	47	1 : 11
B. X. G.P. 1070	1	14	45	1 : 3
B. XIV.	1	12	40	1 : 3·3

The following belong to the most difficult growing viruses, and would yield a result similar to Virus 4. The comparison shows their increased luxuriance on glycerin serum after growing for a few generations on that medium.

B. XVIII.	1	17	27	1 : 1·6
B. XII.	1	5	10	1 : 2

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DESCRIPTION OF THE CHARACTERS OF THE VIRUSES ON THE DIFFERENTIAL MEDIA.

Bovine Viruses.

Virus B. I.

Mediastinal Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Cow 44	17th	Moderately thick uniform grey layer.	No growth occurred until the end of the 2nd week, when a few minute colonies appeared. At the end of the 3rd week the colonies were more numerous but discrete. At the end of the 4th week the colonies had coalesced and covered the whole surface with a grey layer.	The final pellicle was thin uniform grey and semi-transparent with fine whitish lines. After several broth subcultures a rather thicker translucent pellicle with fine wrinkling was formed.	No growth was visible during the first 3 weeks. At the end of the 4th week there were very numerous minute discrete grey colonies scattered over the surface. These increased in size, forming thin grey filmy colonies, some with opaque central foci; these subsequently coalesced in patches.	At the end of 3 weeks minute grey points were seen with a hand lens. In 10 weeks there were moderately numerous small raised colonies of various sizes.

Other strains of this virus—G.P. 14, Cow 40, Calf 106, Baboon 66, Pig 16, Pig 96, and Pig 98—which were tested on the differential media, closely resembled Cow 44.

In the culture obtained from Baboon 8, fed with the milk of Cow 64 (Virus I.), a decided increase of luxuriance has resulted. This increase was maintained after passage through a second baboon (No. 64).

Virus B. II.

Lung.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Heifer 66.	13th	Uniform grey layer with a few raised white colonies.	Growth at the end of 4 weeks was equal to that on pure serum. There was no difference in character with the exception that the small white colonies on the surface were more raised and numerous.	The culture from Heifer 66 was not grown on broth, but in four other strains of this virus the type of pellicle was identical. That from Heifer 100 is described. Growth was slow in beginning: in 3 weeks there was one delicate grey island. In 5 weeks the surface was covered with a delicate grey transparent pellicle composed of small islands speckled with opaque greyish-white foci. In 8 weeks all the growth had sunk.	At the end of the second week there was a thin uniform grey layer with numerous small raised colonies superimposed. A slight increase was noted at the end of the 4th week after which there was no further change.	Very minute discrete greyish-white colonies were first visible at the end of the 4th week. These increased to a very slight extent subsequently.

Cultures raised from six other strains of this virus: Bull 22, Heifer 100, Calf 134, Calf 178, Guinea-pigs 537, 172, 1322 and 1735 agreed very closely in cultural characters with that isolated from Heifer 66.

Guinea-pig 1735 was the 24th of a series of guinea-pigs the first of which was inoculated with the original material of Virus II. The virus was kept going in guinea-pigs to see if any modification by prolonged residence in these animals could be produced. The result showed not the least change in cultural characters.

VIRUS B. III.

MEDIASTINAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct.	15th.	Uniform grey layer of moderate thickness.	There was no growth during the first 2 weeks. At the end of the 3rd week the surface was covered with minute grey colonies. These increased a little in size but remained discrete.	No growth has been obtained on broth. On glycerin gelatine the surface became covered with transparent filmy islands. On an asparagin medium (2 per cent. glycerin) two-thirds of the surface was covered with a delitate grey pellicle in 7 weeks.	At the end of 5 weeks there was no growth. In 10 weeks there were two flat grey colonies with opaque centres.	In 4 weeks there were two minute grey colonies. These increased to four in the 5th week. In 10 weeks the four colonies were rather less than a pin-head in size, creamy in colour and surrounded by a group of very minute grey colonies.

Two other strains of this virus, Monkey 64 and Calf 214, have been investigated, and gave similar results, except that more numerous colonies appeared on glycerin agar in the case of Monkey 64.

VIRUS B. IV.

MESENTERIC GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct.	29th.	Uniform layer of moderate thickness in 3 weeks, with numerous raised white colonies.	In 14 days there was no growth. At the end of 3 weeks the surface was covered with numerous minute grey and greyish white colonies. In the fourth week these coalesced and formed a complete layer, covered with raised white colonies. This increased to a moderate thickness in the sixth week and appeared almost equal in amount to that on serum alone.	On serum and broth, no pellicle was formed at the end of 5 weeks; there were several small yellowish nodules floating on the surface. After subculture for several generations on gelatine broth, a growth was obtained on transference to broth. The type of pellicle produced was thin grey and translucent, with delicate wavy whitish lines.	No growth was obtained with this generation of culture. Several other attempts on different agar media with earlier generations also failed to produce growths.	At the end of 3 weeks minute grey points were seen, which in 3 months showed no change. An earlier generation (17th) showed half-a-dozen opaque white granules at the end of 8 weeks. At the end of 3 months there were 26 colonies of all sizes up to that of a pin-head, slightly creamy in colour.

Cultures of this virus were isolated after passage through different species of animals, namely, Chimpanzees, 4. 6. 8. 10. 16. Baboons, 22. 30. 50. 62. Dogs, 26. 54. 56. and 74. Cat, 12. Rabbit, 5. Calf, 142. Cow, 172. The results on the differential media with these strains closely resembled the above: the only difference was that some of the strains produced discrete colonies on glycerin agar after several weeks. The culture from Dog 18 was quite different from the original virus.

Dog 18.	5th. Broth 6th and 7th.	Thin uniform layer at the end of 3 weeks.	At the end of the first week there was a uniform layer thicker than on serum alone. In the second week it was twice as thick and at the end of the fourth week there was a very thick opaque white finely wrinkled growth.	A bottle of broth sown with the pellicle from the condensation water of a serum tube, showed a single opaque white island in 4 weeks. This was subcultured on broth and in 4 weeks there was a thick opaque white, slightly wrinkled pellicle, covering about a quarter of the surface; growth then ceased.	At the end of the second week, there was a uniform grey layer slightly wrinkled. In the fifth week this had become thick and opaque with well-marked wrinkling of the surface.	In 2 weeks, there was a greyish growth composed of very minute colonies closely aggregated. These increased, becoming slightly yellow and at the end of the seventh week there was a raised opaque granular layer of a yellow colour.

VIRUS B. V.

MESENTERIC GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Heifer 80	12th Broth 19th	Uniform layer of moderate thickness at the end of 3 weeks.	At the end of the 1st week there was a moderately thick grey layer rather better than on serum alone. During the 2nd week the growth became thicker and covered with small white elevated colonies. Growth continued during the 3rd week, the colonies increasing in size. At the end of the 6th week there was a thick greyish white layer, better than on serum, with a moist roughened surface and a slightly wrinkled margin.	In 14 days the surface was covered with a thin uniform translucent grey pellicle already with a tendency to sink.	There was a uniform grey layer at the end of the 1st week. Steady increase took place until the end of the 5th week, when the surface was covered with a grey ground glass layer with fine wrinkling round the edges. At the end of 8 weeks there was very little further increase.	Growth was visible in 11 days as numerous minute grey colonies. In the 5th week there was a layer composed of minute grey colonies. At the end of the 7th week a slight increase was indicated by a few of the colonies becoming a little larger than the rest, being white and slightly raised. No further progress was noticed.

Cultures were also isolated from G.P.'s 711 and 812 inoculated with the original material and were similar to Heifer 80. On 5 per cent. glycerin broth with G.P. 812, the surface was covered in 14 days with a grey translucent pellicle, dotted over with opaque white foci and several warty nodules.

VIRUS B. VI.

MEDIASTINAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	14th.	Uniform layer of moderate thickness in 3 weeks.	No growth was evident until the 3rd week, when the surface was covered with minute grey colonies coalescing in parts. At the 6th week the colonies had fused together, forming an almost continuous thin grey layer.	In 7 weeks there were numerous small grey islands over the surface and a circular patch one and a half inches in diameter rather thicker and speckled with white foci. The growth subsequently sank.	No change was observed until the 6th week, when two minute white colonies were seen. There was no increase at the end of 12 weeks. In the 18th generation no growth could be seen at the end of 4 weeks.	One minute greyish white colony was seen at the end of the 5th week. A few more appeared in the 6th week. 7th week no change.
G.P.1015 (Inoculated with original material)	7th.	Uniform layer.	Uniform layer in 3 weeks identical with that on pure serum.	No growth was obtained.	At the end of the 2nd week there were small sparsely scattered discrete granular colonies. Over part of the surface a thin grey growth developed in between the colonies. At the end of the 11th week there was this patchy grey layer not increased in thickness and numerous small colonies with grey filmy margins.	In the 2nd week there were a few whitish colonies, increasing slightly up to the 5th week. At the end of the 12th week the surface was almost covered with minute pearly-white colonies.

A culture isolated from Calf 216 resembled the direct culture.

Cultures were also obtained from G.P.'s. 1014 and 1015 inoculated with original material; both of these grew distinctly better than the direct culture and were identical with each other. A culture obtained from Calf 240 inoculated with culture of G.P. 1015 had the same characters as the original G.P. cultures.

VIRUS B. VII.
MESENTERIC GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	12th	Uniform grey layer.	Uniform layer in 3 weeks identical in character and amount with that on serum.	In 3 weeks the surface was covered with a very thin grey pellicle which showed no further change. The growth on 2 per cent. glycerin gelatine was identical in appearance excepting that the pellicle was dotted with opaque white foci.	At the end of the 1st week there was a slight initial grey growth, but no further progress until the 3rd week. At the end of the 4th week there was a grey layer not quite uniform. In 5 weeks it was rather thicker and greyish white in parts, due to small confluent colonies. At the end of 2 months delicate grey colonies had appeared in the thinner patches.	Minute grey colonies were visible in the 3rd week. There had been no change 11 weeks later.

Cultures were also isolated from G. P.'s 1016 and 1017, both inoculated with original material. The cultures were identical with Virus VII. direct, but a rather better growth was obtained with G.P. 1017 on potato.

VIRUS B. VIII.
MESENTERIC GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	15th	A moderately thick layer in 3 weeks.	There was a uniform growth a little less than on serum in the 1st week. In the 3rd week the growth became whiter and raised in parts, but on the whole less than on serum. In 6 weeks numerous small raised colonies appeared on the surface.	Inoculated from a serum tube on to a flask of 2 per cent. glycerin broth. In 14 days there was thin grey translucent pellicle speckled with whitish foci, covering about $\frac{1}{3}$ of the surface. In 4 weeks the surface was covered with a greyish translucent membrane studded with raised opaque white colonies.	In 7 days there was a thin uniform grey ground-glass layer, which increased in thickness slightly until the 3rd week. In the 4th, 5th and 6th weeks there was no change. At the end of 10 weeks the surface was studded with minute whitish colonies.	In 14 days there was a greyish layer composed of minute colonies. In the 4th week it was a little thicker and greyish-white, with no subsequent increase.

A culture isolated from Dog 102, fed with culture of original material, showed no alteration.

VIRUS B. IX.
MEDIASTINAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct.	14th.	Thin uniform grey layer not increasing after the 2nd week.	At the end of 7 days there was a grey layer with white points decidedly better than on serum alone. During the 2nd and 3rd weeks the growth became thicker and the colonies whiter and more raised. At the end of the 4th week there was a greyish-white moist layer, at least twice as thick as on serum alone.	On an asparagin medium the surface was covered with a thin grey membrane with a few whitish points. On 5 per cent. glycerin broth in 14 days the surface was partly covered with fairly thick opaque white islands, which sank a few days later.	There was a slight increase at the end of the 1st week. In the 2nd week there was a uniform grey ground-glass layer. Increase took place until the end of the 6th week, when there was a moderately thick grey granular layer, thicker and whiter in places.	At the end of the 4th week there was a thin greyish white layer on the surface of the potato. This increased very little subsequently.

Strains of this virus have been isolated from 15 different animals, including the chimpanzee, monkey, baboon, dog, cat, pig, goat, mongoose, hedgehog, rabbit and guinea-pig; with one exception, that of Dog 50, these have reproduced the characters of the original virus, the pellicle formed on the surface of broth, though varying in thickness, exhibiting the same marked tendency to sink.

VIRUS B. IX.—*continued.*

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Dog 50 through G.P.1708	3rd	Thick uniform greyish - white layer.	In the 1st week the growth was thicker than on serum. At the end of the 2nd week the layer was thick, creamy and markedly wrinkled. Subsequently the wrinkling became coarser and the colour more yellow.	At the end of 6 weeks there was a thick opaque pellicle, creamy in colour, with a finely granular surface. No tendency to sink.	Growth took place from the outset and in 6 weeks the surface was covered by a thick opaque, yellow, coarsely wrinkled layer.	At the end of the 1st week there was a greyish-white layer. This increased until at the end of 6 weeks there was a thick raised yellow growth with a granular surface.

A culture was also isolated from Dog 50 through G.P. 1707; its cultural characters were identical with those of G.P. 1708.

VIRUS B. X.

MESENTERIC GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
G.P.1070 (Inoculated with original material)	20th Broth 16th	Moderately thick grey layer with a few small whitish colonies.	At the end of the 1st week there was a layer rather thicker than on serum alone. It increased in thickness until the end of the 4th week when there was a greyish white finely wrinkled layer about twice as thick as on serum without glycerin.	In 14 days the surface was incompletely covered by several uniform grey translucent islands. There was slight thickening subsequently, the pellicle remaining translucent. In 4 weeks the growth had sunk.	In 7 days there was a grey translucent layer covered with small white colonies. The growth increased in thickness until the 4th week, becoming greyish white and opaque in patches with fine wrinkling on the under surface.	In 6 weeks numerous minute white colonies were observed. There was considerable increase until the 10th week when the colonies in part coalesced to form a patch of grey growth, and others became raised and slightly yellow.

A culture was isolated from the supramammary gland through G.P. 1072 and was identical in cultural characters.

VIRUS B. XI.

MEDIASTINAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Rabbit 66 (Inocu- with original material)	11th	In 3 weeks there was a thick uniform grey layer.	At the end of the 1st week there was a greyish white layer with small colonies, thicker than on serum alone. During the 2nd and 3rd weeks the growth became thicker and more raised and in 4 weeks there was a layer covered with greyish white colonies at least twice as thick as on serum alone.	In 14 days the surface was nearly covered by several grey translucent islands. In 28 days the surface was completely covered with a greyish translucent pellicle covered with white raised foci. There was very little further thickening but the growth showed no tendency to sink.	In 7 days there was a grey translucent layer with whitish opaque foci. This increased until the 3rd week when there was a greyish white ground-glass layer with small white colonies.	A thin whitish layer was formed in 3 weeks not increasing subsequently.

A culture was also isolated from Rabbit 65 and yielded similar results.

Virus B. XII.

Lung and Mediastinal Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct (Lung)	16th	Uniform layer of moderate thickness with raised white colonies on the surface.	No growth occurred until the end of the 3rd week when minute colonies could be seen with a lens. During the 4th week numerous small grey colonies became visible. At the end of the 6th week there were numerous flat grey colonies mainly discrete and several raised warty colonies of various sizes.	At the end of 6 weeks there were numerous small grey transparent islands which showed no subsequent change.	No growth had occurred at the end of the 9th week.	In 5 weeks there were a few minute whitish raised colonies. In 10 weeks there were fairly numerous raised whitish colonies less than a pin-head in size with grey filmy margins.
Rabbit 98 (Inoculated with the Mediastinal Gland)	17th	Uniform grey layer of moderate thickness.	Growth took place from the outset in the form of a uniform grey layer which progressed at the same rate as on serum alone. At the end of 4 weeks the growths were identical in character and amount.	On an asparagin medium the surface was covered in 5 weeks by a thin grey pellicle speckled with whitish foci.	A very thin grey layer was formed during the 1st week. Slow but definite progress was made and at the end of the 4th week there was a thin translucent layer with a finely granular surface.	Very numerous minute grey colonies were visible in the 4th week.

A culture was also isolated from G.P. 1159 inoculated with original material; this culture yielded similar results to that isolated direct, with the exception that on glycerin agar a few grey colonies appeared during the 9th week.

Virus B. XIII.

Mediastinal Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Rabbit 116 (Inoculated with the original material)	11th	Thin uniform layer.	There was no growth during the first 2 weeks. At the end of the 3rd week there were fairly numerous discrete whitish colonies. At the end of the 4th week the colonies had increased in size, the largest being about the size of a pin head. Around each colony there was a delicate grey marginal expansion which increased until the 6th week, the colonies however remaining discrete.	At the end of 6 weeks there were several delicate grey speckled islands. The surface eventually became covered with a thin semi-transparent pellicle, partly uniform and partly reticulated.	At the end of the 4th week very numerous minute delicate grey colonies could be seen with a hand-lens. In 6 weeks these had increased in size with raised opaque centres and had coalesced in parts.	At the end of the 9th week there was no growth visible. After 3 months fairly numerous minute grey colonies were seen on the centre of the potato.
G.P.1269 (Inoculated with the original material)	13th	Similar.	Colonies were not visible until the 4th week. They had fused together at the end of 6 weeks, forming a thin grey layer.	In 2 months the surface was almost covered by a fine lace-like pellicle which did not increase subsequently.	No growth until the 9th week when two small white raised colonies were visible.	There was no growth until the 9th week, when two small whitish raised colonies were seen. In 3 months these had become conical and faintly yellow. At the end of 4 months the colonies were warty and numerous minute grey ones had appeared.

Virus B. XIV.

Pleura.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct. ('Grapy' masses from Pleura)	13th.	Uniform growth of moderate thickness at the end of the 3rd week.	At the end of the 1st week the growth was a little thicker than on serum alone. After the 3rd week the growths were about equal in amount, but that on glycerin serum was moister, and the surface was roughened by small, raised, white colonies. At the end of the 5th week the growth was thicker than on serum, and the colonies had become yellowish in colour.	On an asparagin medium the surface was covered in 5 weeks with a very delicate grey pellicle, composed of numerous small islands, which, later, sank to the bottom.	Growth during the 1st week was in the form of a thin, grey layer with small, white nodular colonies. During the next 7 weeks steady increase took place, the colonies becoming more raised. At the end of the 9th week there was a fairly thick, greyish-white growth of unequal thickness, covered with small raised colonies. No tendency towards wrinkling.	Numerous minute grey colonies appeared during the 2nd week. During the 3rd week a few became whiter and slightly raised. At the end of the 5th week there was a grey layer, consisting of very small raised colonies. There was no subsequent change.

A culture, isolated from G.P. 1271, inoculated with original material, covered the surface of broth (5 per cent. glycerin) in five weeks with a thin, uniform, translucent grey pellicle, which showed no tendency to sink. On the solid media the culture resembled that obtained direct.

Virus B. XV.

Mesenteric Gland and Mediastinal Gland.

Strain.	Generation	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct. (Mesenteric Gland)	7th. Agar 16th.	Moderately thick grey layer, showing a few white colonies on the surface in the 3rd week.	No growth occurred until the 4th week, when a few scattered grey colonies appeared. At the end of the 6th week the colonies had developed grey filmy margins, coalescing near the condensation water where the colonies were most numerous.	In 6 weeks the surface was covered with loosely aggregated delicate grey islands, speckled with whitish points. Later, the growth all sank. A growth similar in character was obtained on a 2 per cent. glycerin asparagin medium.	There was no growth until the end of the 4th week, when numerous minute grey colonies could be seen. At the end of the 5th week these had coalesced and their centres had become raised and opaque.	Minute greyish-white colonies were just visible at the end of the 5th week. In 7 weeks they were so numerous as to form a greyish-white layer. In 12 weeks a few colonies had become slightly raised; beyond this there was no increase.
G.P. 1348 (Inoculated with the Mediastinal Gland)	5th, Broth 8th.	Similar.	A few scattered minute colonies were seen at the end of the 3rd week. In the 4th week the colonies were small and greyish-white with grey margins.	On a 2 per cent. asparagin medium in 4 weeks, over half the surface was covered with an almost invisible grey network, most of which, at the end of 6 weeks, had sunk to the bottom.	No growth obtained.	In 5 weeks there was one small raised colony. In 7 weeks these were slightly raised, with spreading margins, and numerous very minute grey ones had appeared. In 3 months there were 4 pinhead-sized colonies with grey filmy margins, and numerous smaller colonies over the surface.

A culture was also isolated from G.P. 1347, inoculated with the original material at the same time as G.P. 1348 and showed identical characters.

VIRUS B. XVI.

BRONCHIAL AND MEDIASTINAL GLANDS.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct. (Bronchial Gland)	10th.	Uniform grey layer of moderate thickness.	Numerous minute grey colonies were visible in the 3rd week. These increased slightly in size up to the 4th week, growth then ceasing.	Inoculated on to 5 per cent. glycerin broth from a serum tube. About one third of the surface was covered with delicate grey islands in a month. One of these islands increased, forming at the end of 3 months a greyish white lace-like pellicle 1½ inches in diameter. On a 5 per cent. glycerin gelatine bottle sown at the same time an almost invisible grey film covered the surface in 11 weeks.	No obvious growth in 11 weeks.	There were minute greyish-white colonies visible at the end of the 3rd week. In 5 weeks these were fairly numerous. Similar colonies and numerous minute grey ones could be seen with a hand-lens. There was further increase until the 9th week; the final growth being a thin grey layer surrounding greyish-white raised colonies.
	12th.	Similar.	At the end of the 1st week there was a thin grey layer equal to that on serum. The layer showed no subsequent thickening but numerous discrete grey colonies appeared in the 2nd week, becoming raised and greyish-white in the 6th.	The surface was covered in 15 days partly with a very thin grey layer and partly with small grey islands. In 4 weeks the growth had sunk to the bottom.	At the end of the 1st week there was a thin grey layer with greyish-white points. The points developed into greyish-white colonies, the initial layer showing very little increase.	No trial.
G.P.1399 (Inoculated from the Mediastinal Gland)	3rd.	Uniform grey layer of moderate thickness.	In one week there was a very thin grey layer increasing until the 3rd week, becoming thicker in parts than on serum. In a second pair of tubes the final growth was rather better than on serum alone.		In 7 days there was a thin translucent grey layer which became a little thicker along the margins in 14 days. At the end of 6 weeks numerous delicate grey colonies had developed, some of which had become slightly raised.	At the end of 4th week there were numerous minute greyish-white colonies. In six weeks they had become more numerous. There was very slight increase in size up to the 3rd month.

Virus B. XVII.

Mediastinal Gland and Lung.

Strain.	Generation.	Serum.	Glycerin Serum	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct (Mediastinal Gland)	8th.	Thin uniform grey layer.	There was no change until the 3rd week when the surface was covered with minute discrete grey colonies. These increased a little in size but remained discrete.	The pellicle from the surface of the condensation water of a serum tube was sown on to 5 per cent. glycerin broth and a 2 per cent. glycerine asparagin medium. No growth occurred on the former: the surface of the latter was covered in 8 weeks with a delicate grey transparent membrane with fine white curved lines.	At the end of 3 months there was practically no growth.	A few minute grey points were seen with a lens at the end of the 3rd week. In 3 months there was no change.
G.P.1411 (Inoculated with the Lung)	3rd	Thin uniform grey layer.	There was a uniform layer equal to that on serum at the end of the 1st week. The growths were practically identical at the end of 6 weeks.	The condensation water pellicle sown on a 5 per cent. glycerin broth produced no growth.	No growth was obtained.	Minute grey points were visible with a hand-lens in 7 weeks. In 9 weeks these had formed definite raised colonies with grey filmy margins.

Virus B. XVIII.

Bronchial Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct.	3rd. Agar 11th. Broth 10th.	Uniform layer of moderate thickness.	Numerous minute grey colonies were visible in the 4th week. Subsequently the colonies developed delicate grey filmy margins which had coalesced at the end of the 6th week.	At the end of the 6th week there was one small grey speckled island. Four weeks later there were two small grey islands speckled with opaque white foci.	There was no growth during the first three weeks. At the end of the 6th week there was a thin grey granular patch of growth formed by coalescing colonies some of which had become raised and whitish.	No growth occurred until the 8th week when two minute colonies appeared. At the end of the 12th week there were three slightly raised creamy colonies and several minute grey ones. In 16 weeks there were fairly numerous discrete colonies ranging from a point to a pinhead in size.
Rabbit 160. (Inoculated with the original material)	5th.	Thin grey glazed layer.	Numerous minute colonies appeared in the 3rd week. These extended superficially and in 6 weeks had coalesced to a large extent forming a thin incomplete layer.		No growth.	In the 5th week a patch of minute grey colonies appeared. In the 7th week one was raised and creamy and in 10 weeks the small colonies had become confluent.

VIRUS B. XIX.
BRONCHIAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	5th. Agar 14th.	Uniform grey layer of moderate thickness with whitish colonies on the surface.	No growth occurred during the first 2 weeks. At the end of the 3rd week the surface was covered with very numerous minute grey colonies coalescing to form almost a complete layer at the end of the 4th week. In 6 weeks there was a complete layer studded with greyish white raised colonies. The amount of growth then appeared greater than on serum alone.	A few small grey islands formed on the surface in 3 weeks.	No growth was obtained until the 4th week when the surface became covered with very numerous minute grey colonies. In the 5th week these had increased in size but remained exceedingly thin and delicate.	In 4 weeks numerous minute white colonies were visible over the surface. In 6 weeks a few became slightly raised. There was no subsequent change.
G.P.1470 (Inoculated with the original material)	6th.	Similar.	During the first 3 weeks there was no growth. At the end of 6 weeks there were fairly numerous raised greyish-white discrete colonies ranging in size from a point up to nearly a pinhead.	No growth during the first 3 weeks. At the end of 6 weeks there were several small whitish nodules floating on the surface.	No growth during the first 3 weeks. At the end of 6 weeks there was a thin grey layer composed of delicate confluent colonies.	No growth at the end of 6 weeks.

VIRUS B. XX.
BRONCHIAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct.	8th. Broth 14th.	At the end of 3 weeks there was a uniform growth of moderate thickness with a few raised white foci.	At the end of the 1st week there was a uniform grey layer, equal in thickness to that on serum, but with more raised foci on the surface. In the second week the growths were still equal. At the end of the 4th week the growth was distinctly thicker than on serum and was moist and greyish-white.	In 2 weeks the surface was practically covered with a delicate grey pellicle in part composed of islands, with scattered opaque white foci. At the end of the 3rd week the growth had sunk.	In 7 days there was a thin uniform grey layer increasing in thickness slightly up to the end of the 3rd week with the development of a few raised colonies. At the end of the 6th week there was a greyish-white granular layer with a few opaque white colonies forming a small warty patch.	A few whitish points were visible in 14 days. In 5 weeks there were fairly numerous minute greyish white colonies. In 8 weeks there was an increase in the number of the colonies which remained minute, some being visible only with a hand lens.
G.P. 1471. (Inoculated with original material)	5th	Uniform grey layer.	In 7 days there was a uniform grey layer, thinner than on serum: this increased slowly, parts becoming raised greyish - white moist and ribbed, parts remaining quite thin and transparent.	In 18 days there were three or four small grey translucent islands, which subsequently sank.	In 7 days there was a thin uniform ground-glass layer which had attained a moderate thickness at the end of the 3rd week, showing a slight fine wrinkling on the under surface. There was very little subsequent increase.	In 14 days there was a thin grey layer on the centre of the potato, which at the end of the 4th week became greyish-white and slightly raised. Growth then ceased.

A culture was also isolated from G.P. 1472, inoculated at the same time and with the same material as G.P. 1471 : this culture gave practically identical growths on the solid media, but on broth formed a uniform transparent grey pellicle covering the surface at the end of 6 weeks.

VIRUS B. XXI.
BRONCHIAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	5th	Moderately thick uniform grey layer.	A few very minute colonies were visible in 14 days. At the end of the 3rd week there were numerous grey colonies closely aggregated in places. In 6 weeks there was very little further change	No growth obtained. On a second bottle several small speckled islands were formed.	In 8 weeks the only change was a slight thickening along one side of the slope of the inoculated material.	In 4 weeks minute grey points were seen. In 6 weeks discrete greyish-white colonies scattered over the surface were distinctly visible. In 8 weeks there were very numerous small slightly raised greyish-white colonies with grey filmy margins.

VIRUS B. XXII.
(?) MEDIASTINAL GLAND.

Strain.	Generation.	Serum	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
G.P.1508 (Inoculated with the original material)	4th.	Uniform grey layer of moderate thickness.	In the 1st week the layer was not so uniform and rather less than that on serum alone. In the 2nd and 3rd weeks the surface of the growth became whiter in parts with fine wrinkling the amounts of growth being about equal on the two media.	In 12 days there were a few delicate grey islands on the surface and growth was starting along the serum. In 3 weeks the surface was covered with an almost transparent pellicle.	In 7 days there was a thin transparent layer which increased slightly during the next 3 weeks. In 6 weeks there was a thin grey translucent layer varying slightly in thickness.	A slight increase of the inoculated material was noticed in 6 weeks.

A culture raised through G.P. 1509 showed similar characters, very little growth being obtained on potato, but a slightly thicker pellicle on broth.

Dog 72	3rd.	Moderately thick grey glazed layer.	In the 1st week the layer was thicker than on serum without glycerin and greyish white. At the end of four weeks there was a thick moist greyish white layer with fine wrinkling.	The surface was covered in 4 weeks with a uniform grey translucent skin with whitish foci. There was no further thickening.	In 14 days there was a moderately thick dry greyish white layer. In 4 weeks the surface of the growth showed a fine raised wrinkling. There was no further thickening but subsequently opaque creamy colonies developed.	At the end of the 1st week there was a thin grey layer. From this after several weeks raised irregular warty colonies grew.

VIRUS B. XXIII.
LUNG.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	5th	Thin uniform grey layer in 3 weeks.	In 7 days there was a uniform grey layer distinctly better than that on serum. In 14 days the growth was still better and in 3 weeks there was a moist greyish white layer of unequal thickness apparently double the amount of that on serum. No further change was noted.	The surface of the broth was covered with a number of semi-transparent grey islands not forming a complete pellicle. With the 8th generation the surface was in 3 weeks incompletely covered by a pellicle so delicate as to be almost invisible.	In 7 days there was a thin uniform grey ground-glass layer which increased a little in thickness until the end of the 3rd week. There was then a grey translucent layer slightly thicker and opaque in parts.	The surface was covered in 7 days with a greyish growth composed of minute colonies. These slowly increased in size, a few at the end of the 4th week becoming slightly raised—one attaining the size of a pin-head. At the end of the 10th week there was a greyish white layer with a granular surface.

A culture was also isolated from G.P. 1,534 inoculated with the bronchial gland; this culture resembled that obtained direct, but on broth formed a uniform translucent pellicle which covered the whole surface and began to sink at the end of 6 weeks.

Virus B. XXIV.

Lung and Bronchial Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct ('Grapy' growths from Lung.)	4th.	Uniform layer of moderate thickness.	There was no growth visible until the end of the 3rd week when very numerous minute discrete colonies were observed with a hand lens. At the end of the 4th week they formed a thin grey layer. There was no further change.	No complete pellicle was formed, the surface of the broth after 6 weeks showing only a few very small islands. Some transparent, others greyish-white and granular.	During the first 6 weeks there was no growth. At the end of the 10th week there were three flat grey colonies — the largest about 2 mm. with raised opaque centres.	In 4 weeks minute grey colonies were seen with a hand lens and one or two visible to the naked eye. In 5 weeks the surface was covered with very numerous greyish-white colonies forming a thin layer. There was no subsequent change.
G.P. 1548 (Inoculated with the Bronchial Gland.)	4th.	Uniform layer of moderate thickness at the end of the 3rd week.	Numerous minute colonies were just visible at the end of the 3rd week. In 4 weeks the surface was covered with numerous grey colonies which at the end of 6 weeks had grown larger and to a great extent coalesced.	At the end of six weeks there were numerous transparent islands and opaque white fragments of growth: the surface was not covered.	No growth visible in 10 weeks.	In 5 weeks there were a few minute transparent grey colonies with greyish-white centres. These colonies did not increase in size.

Cultures of this virus were raised direct from the lung and bronchial gland and from the bronchial gland through G.P. 1548. The culture isolated from the bronchial gland was identical with those described above.

Virus B. XXV

Pleura.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	9th	Thin uniform, grey layer not increasing after the 2nd week.	In the 1st week there was a uniform grey layer thicker than on serum alone. At the end of the 2nd week the growth was greyish-white and slightly ribbed round the margins. At the end of the 4th week the layer was two or three times as thick as on serum alone, with a greyish-white roughened surface.	In 11 days the surface was covered with a semi-opaque grey pellicle, climbing up the sides of the bottle. It subsequently began to break up and showed a tendency to sink without further thickening.	In 7 days the surface was covered by a grey layer of unequal thickness. At the end of the 2nd week it was quite thick and opaque in streaks and showed a tendency to wrinkle, which became more definite at the end of the 3rd week, after which there was no change.	Growth took place from the outset in the form of a thin grey moist layer. This remained unchanged until the 2nd month, when small colonies appeared which subsequently became large, irregular, and yellow in colour.
G.P.1607 (Inoculated with the original material)	5th	Similar	Similar.	In three weeks the surface was covered by a thin grey translucent pellicle which did not thicken.	In the 1st week there was a thin grey layer with small whitish patches. This increased until the end of the 3rd week, being for the most part translucent but thick and opaque in patches.	Thin grey layer with the formation of raised white colonies at the end of 5 weeks.

VIRUS B. XXVI.

BRONCHIAL GLAND.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	7th	Uniform grey layer of moderate thickness.	No growth occurred until the 3rd week, when the surface was covered with minute grey colonies. These coalesced in the 4th week, forming a thin grey layer which did not increase.	In 8 weeks ¾ of surface was covered with delicate grey islands speckled with opaque foci and small warty nodules. At the end of the 13th week there was no change except for some increase of the whitish nodules. There was no tendency to sink.	No growth occurred in 7 weeks. In 10 weeks there were 2 raised irregular colonies, the largest about the size of a pinhead. In the second generation after 5 weeks there were several small delicate grey colonies. These spread slightly, remaining thin, with minute opaque centres.	In the 3rd and 4th weeks a few minute white colonies became visible and showed no further increase. In the 2nd generation no growth occurred until the 5th week, when minute white colonies were visible. These increased, and in 3 months were fairly numerous, in size from a point up to 1 mm., with delicate grey margins.

A culture was also isolated through Rabbit 380, which was inoculated with the Original Material; it was identical in characters with the direct culture.

VIRUS B. XXVII.

DIAPHRAGM.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	4th	Uniform grey layer of moderate thickness.	At the end of the 1st week there was a greyish white layer thicker than on serum alone. In the 2nd week it was about twice as thick, and showed at the end of 6 weeks little further change.	In 10 days there was a uniform grey pellicle covering ⅔rds of the surface. In 14 days the surface was completely covered with a greyish white translucent pellicle, with small opaque patches, already showing a tendency to sink. The growth subsequently sank without further thickening.	At the end of the 1st week the surface was covered by an unequal grey layer of moderate thickness in parts. The thicker patches increased, and became slightly ribbed at the end of the 4th week. In the 2nd generation there was formed at the end of the 4th week a greyish-white translucent layer which had slowly increased from the outset.	In the 1st and 2nd weeks there was a thin grey layer which showed no subsequent increase in thickness. At the end of the 11th week there were scattered small raised white colonies growing from the grey layer. In the 2nd generation there was formed in the 1st week a thin grey layer, which increased slightly up to the end of the 3rd week.

VIRUS B. XXVIII.

THORACIC GLAND. (?)

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	4th	Uniform grey layer of moderate thickness at the end of 3 weeks.	A uniform layer was formed in the first week a little thicker than on serum. It became at the end of the 4th week nearly twice as thick as on serum remaining uniform.	In 14 days there was a grey translucent island about one inch square. At the end of 5 weeks there were 4 grey islands covering about one-sixth of the surface. There was no further increase. On a second bottle the result was similar.	At the end of 12 days there was a grey ground glass layer over the surface. This increased until the 4th week, becoming finally wrinkled in parts. There was no subsequent increase.	During the first 2 weeks no growth was evident. At the end of 3 months there was a thin grey layer, with yellowish colonies up to a millet seed.

Virus B. XXIX.

Mediastinal Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	4th	Uniform grey layer of moderate thickness.	Growth took place from the outset and was rather thicker than on serum alone at the end of the 2nd week. At the end of the 4th week there was a grey layer not quite uniform and nearly twice as thick as on serum alone.	In 4 weeks the surface was not quite covered by a grey translucent almost uniform pellicle with minute white foci. This remained unchanged and showed no tendency to sink.	In 14 days there was slight increase where the inoculated material was thickest, and a patch of thin grey growth near the condensation water. Six weeks later there had been very little change.	There was a thin greyish moist layer becoming definite at the end of the 3rd week. There was no further thickening, but at the end of the 6th week there were three small raised white colonies.

Virus B. XXX.

Bronchial Gland.

Strain.	Generation.	Serum.	Glycerin Serum.	Glycerin Broth.	Glycerin Agar.	Glycerinated Potato.
Direct	3rd.	Thin uniform grey layer.	Growth was of the same character as on serum and progressed at the same rate until the end of the 3rd week. Subsequently the layer became a little thicker and moister than on serum and showed raised white foci on the surface.	In 4 weeks the surface was covered with a thin grey translucent pellicle on which are scattered irregular whitish foci.	In 8 days there was a slight increase of the inoculated material. There was a slow increase until the end of the 4th week, a very thin and incomplete grey layer being formed.	No growth.

Plate I.

CULTURES OF TUBERCLE BACILLI ON GLYCERIN AGAR.

Showing the influence of glycerin in increasing cultural luxuriance.

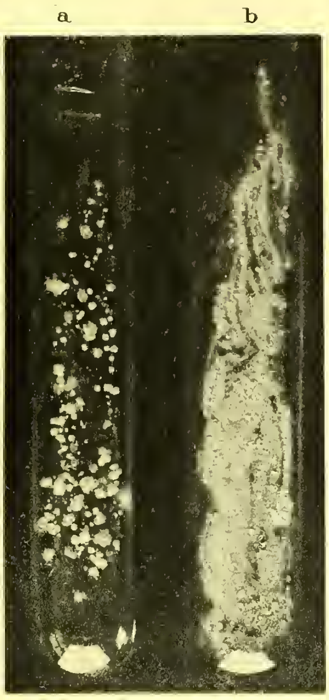


Fig. I.

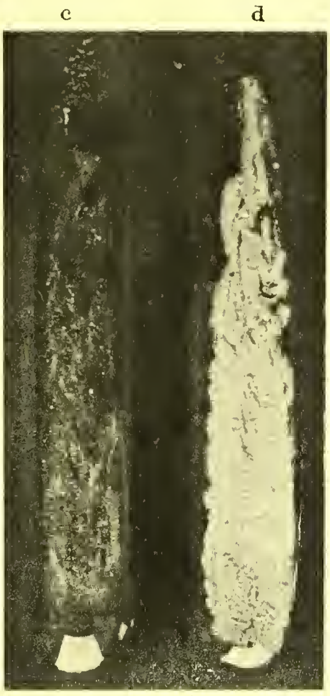


Fig. II.

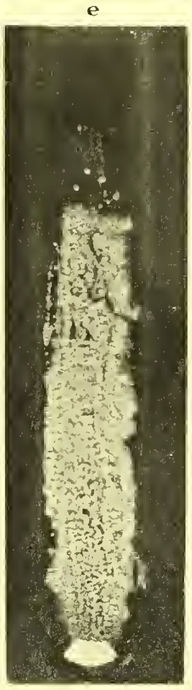


Fig. III.

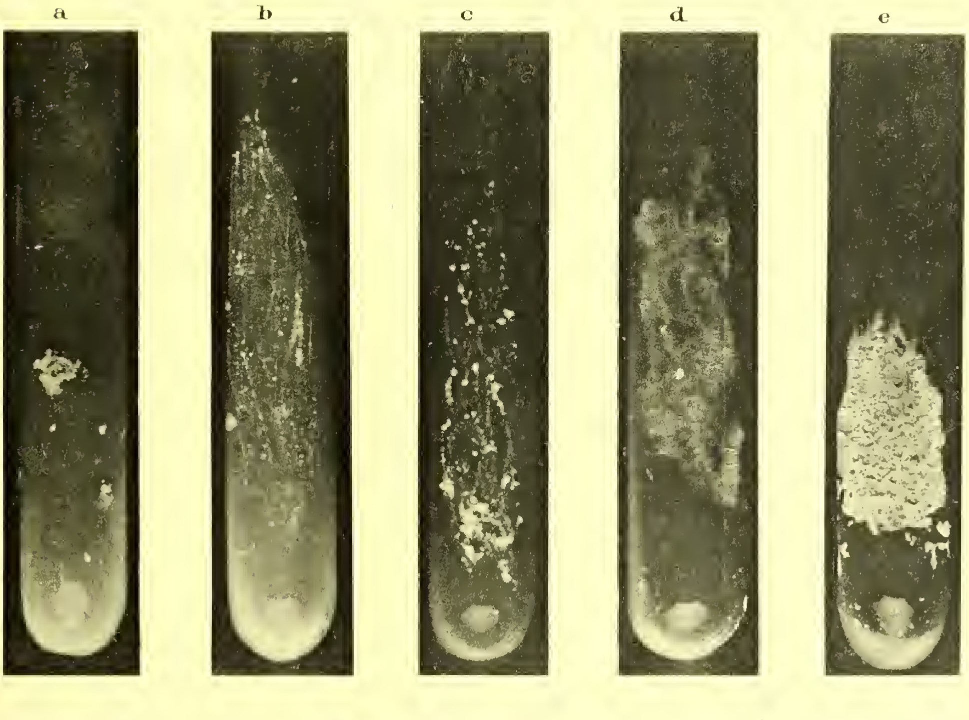
Fig. I. Illustrates the type of growth produced on glycerin agar by bovine bacilli of Class I.
- a. Grown since isolation on pure serum.
- b. Grown for several generations on glycerin serum.

Fig. II. Illustrates the type of growth produced on glycerin agar by bovine bacilli of Class III.
- c. Grown since isolation on pure serum.
- d. Grown for several generations on glycerin serum.

Fig. III. A culture on glycerin agar of an easy growing tubucle bacillus of low virulence, included for comparison.

CULTURES OF TUBERCLE BACILLI ON GLYCERIN AGAR.

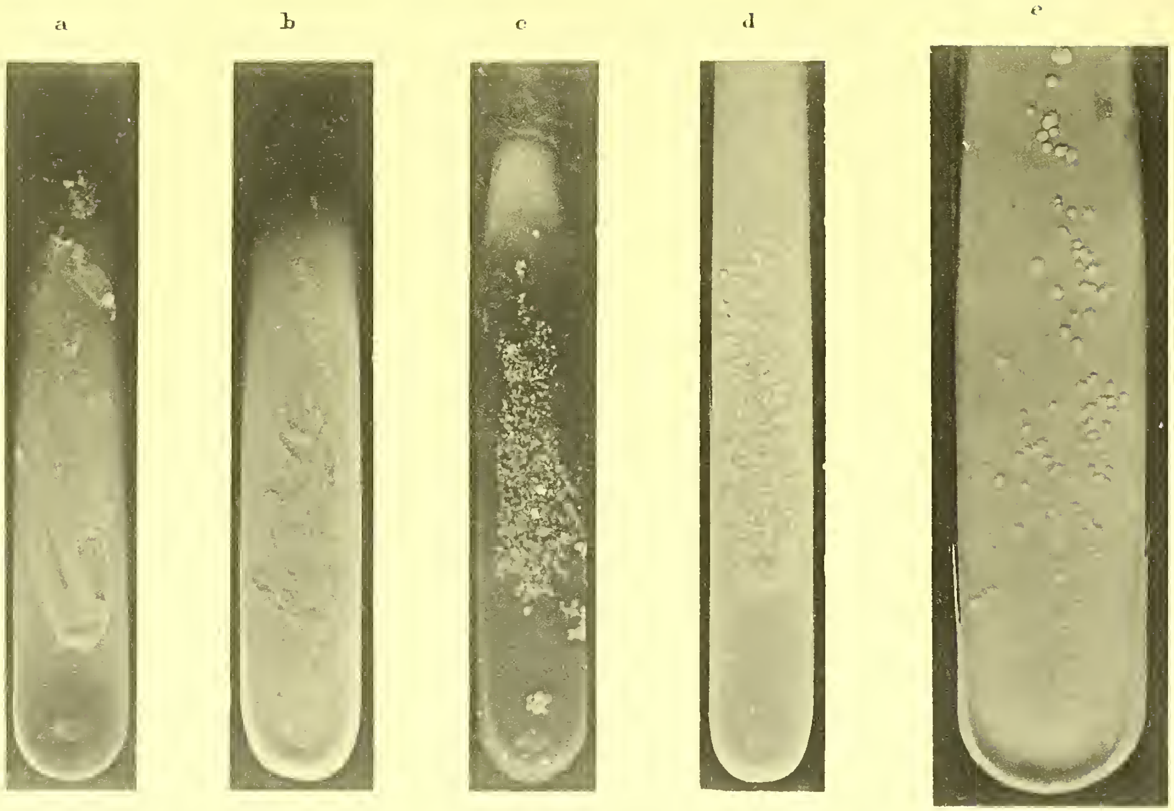
Showing different types of growth.



a. Virus B.I. Strain derived from Heifer 28. (Prescapular gland) through Guinea-pig 540.
b. Virus B.II. „ „ „ the original material, through the Guinea-pig.
c. Virus B.V. „ „ „ Heifer 80.
d. Virus B.IV. „ „ „ Dog 18. (mesenteric gland)
e. Virus B.IX. „ „ „ Dog 50. (Lung)

Plate 3.

PRIMARY CULTURES OF BOVINE TUBERCLE BACILLI
ON VARIOUS MEDIA.



a Culture on Cat serum, 3 months old.
b Culture on Pig serum, 3 months old.
c. Culture on Dog serum agar, 3 months old.
d. Culture on bovine serum + 5% glycerine, 3 months old
e. Culture on the egg medium, 4 months old.

THE CULTURAL CHARACTERS OF THE TUBERCLE BACILLI OBTAINED FROM MAN.

Dr. L. COBBETT.

THE CULTURAL CHARACTERS OF THE TUBERCLE BACILLI OBTAINED FROM MAN.

During the last three and a half years tubercle bacilli from sixty cases of human tuberculosis have been cultivated, and many strains raised, some direct from the human tissues, some from animals injected from them, and others again after the strain had passed through a greater or smaller number of animals of various kinds.

Over 160 of these strains have been examined as to their mode of growth on various media, both in primary culture, and in secondary cultures extending over a varying period of time. Many of the strains have been in continuous cultivation since their isolation up to the present time, some for over three years. Many, too, have been grown in large quantities for the injection of animals, and this has offered an opportunity of *measuring* the relative amount of growth on different media.

Cultures have been made on all sorts of media including the serum of many different animals, and egg, as well as serum, agar, broth, and potato to which 5 per cent. of glycerin has been added. Other media also have occasionally been used, such as glycerin-serum-agar, glycerin-egg, glycerin-serum-broth, and broth without any glycerin. The effects of long continued cultivation on serum with and without glycerin have been observed.

When working with cultures of bovine origin at Walpole Farm, I observed that those which were then under observation would not grow upon glycerin-serum, or grew less readily upon that medium than upon serum to which no glycerin had been added. On being transferred to Blythwood Farm and commencing to work with bacilli of human origin, I observed that the majority grew far more luxuriantly on media which contained glycerin than upon those which did not, while a minority grew like the bovine bacilli which I had cultivated at Walpole. The former were only slightly virulent for the ox and rabbit, the latter were highly virulent for these animals. Further experience, however, showed that some of those strains which were highly virulent for these animals, grew rather better on glycerin-serum than upon serum which contained no glycerin, but not so luxuriantly as those which were only slightly virulent for these animals.

Referring now only to tubercle bacilli of human origin, it may be said that different strains grow very variously on a given medium. On serum and egg the differences are not marked; they are far greater on media which contain glycerin. Thus on glycerin-serum there may be a thick nodular continuous cream-coloured growth, or a thin scarcely visible grey film formed of separate points; there may be no growth at all, or anything between those extremes. On glycerin-agar there is often no growth, often a colourless grey film of small points, and frequently again there is a more or less continuous nodular or wrinkled growth of raised cream-coloured colonies. Sometimes, when the colonies are few and widely isolated, they grow into large and wrinkled pyramids. The growth on broth is equally varied. There may be a partial and delicate grey film, or a thick layer which covers the surface, climbs the side of the vessel and becomes wrinkled. Some cultures form a thin delicate reticulated membrane resembling lace, others, large thick islands, the surface of which is roughened by innumerable projections. In some the growth remains very limited and forms a small thick isolated island, which after a long period of apparent quiescence may send out a thin film which rapidly covers the surface. Experiment has shown that a high degree of acidity increases the tendency to the formation of a thick limited patch of growth, and it may well be that the rather sudden changes in the character of growth just described are due to the lessening of the acidity below a certain unfavourable point. This kind of change has been observed in the growing strains which have no tendency to form acid.

The growth of a given strain on a given medium is variable, conditions not entirely under our control apparently playing a dominant part. On liquid media an accidental shake may cause the commencing film to sink, and growth to become arrested. To encourage growth on liquid media various devices have been tried. A small piece of cotton wool in the broth, just sufficient to rise to the surface, has been found to afford a convenient resting place for the bacilli. But the plan which usually succeeds best is to pour a little glycerin-broth into a serum tube which is already growing well, and when floating growth has appeared on this to transfer it to a flask containing the liquid medium.

In consequence of the uncertainty of growth of a given strain on a given medium, in spite of all efforts to obtain constant conditions, no conclusions as to cultural characters can be drawn except after repeated observations, only the best growths obtained with a given strain on a given medium being taken as typical.

No systematic observations covering all the strains grown here have been attempted. I therefore do not propose to submit a detailed report of my observations on cultures, but will briefly summarise the conclusions to which I have been led.

(1) Tubercle bacilli from man fall into two groups, which present fairly distinct characters of growth.

(2) These characters are determined by *quantity* and *rate* of growth on certain culture media. The ultimate quantity appears to me the more significant and useful, but others have thought that the rate of growth was the most distinctive.

(3) The differences in the quantity of growth—as shown by the thickness, colour and other appearances of the film—produced by typical strains of the two groups, is not marked on pure serum, and no classification according to the cultural characters on this medium would be possible. It is on media containing *glycerin* that the difference becomes marked.

It is perhaps most marked on glycerin-agar and glycerin-broth, but those media are apt to be uncertain, and growth on them at the best is slow. Glycerin-serum appears to me to be more certain, and on it results are more quickly obtained.

(4) The two cultural groups into which human tubercle bacilli fall may be defined as including—

1. All those strains the growth of which in early cultures is little, if at all, aided by glycerin; and
2. Those which grow much more luxuriantly on media to which glycerin has been added.

(5) A rough but useful method of making preliminary observations on the cultural characters of a given strain is to sow it on serum and glycerin-serum and to compare the two growths after three weeks. If the growth is decidedly better on glycerin-serum than upon pure serum, it will certainly belong to the second group. If it is better or not markedly inferior on pure serum it will belong to the first group. This test will not apply, as will presently be explained, to old cultures, or to those which have become accustomed to glycerin media.

(6) The unavoidable variations in our culture media, and the variations in characters of growth which may depend upon them, and also perhaps on other factors which

are not under our control, are such that the characters of a given strain cannot be determined without repeated cultivation; and in forming our judgment *only the best growths on a given medium should be considered characteristic.*

(7) It has been shown elsewhere that tubercle bacilli from man, classified according to their *virulence* for certain animals, fall into two principal groups. It is otherwise with the groups into which the bacilli fall, when classified according to their cultural characters.

Cultural characters go, to a certain extent, hand in hand with virulence. The extreme members of each cultural group differ greatly from each other, but there are others where the difference is less marked. Thus while comparative virulence does not seem to vary among the members of each group, cultural characters do. It has even been doubted whether it is reasonable to make two cultural groups, and whether after all the various strains do not form a series in which each presents minimal differences from its neighbours. It is true that examination of the cultural characters on glycerin-agar of a large number of different strains showed that they formed a continuous series in which there was no gap sufficient to justify a division into two groups. But this, I believe, was, to a great extent, explained by the fact that the strains were not strictly comparable in that they had been a very different length of time under cultivation. And it is an easily demonstrable fact that tubercle bacilli gradually adapt themselves to our artificial culture media and grow better upon them after continued cultivation than when first isolated from the animal.

Bacilli which grow little or not at all on glycerin media when freshly isolated from the living animal body acquire the power of growing on these media after long-continued cultivation. And those which grow fairly well on it at first may acquire a luxuriance of growth after several generations. The power of growing, or of growing better on glycerin media, is acquired to some extent by cultivation on media such as serum which contain no glycerin, but the adaptation is carried further if the strain is accustomed to glycerin itself, by being grown on media which contain it. In this way strains of bacilli which belonged originally to the first group come to be indistinguishable from members of the second group; that is, they are indistinguishable by their cultural characters alone; their virulence has not altered.

(8) For these reasons a classification of tubercle bacilli according to their cultural characters can only be made by comparing strains which have been the same length of time under artificial cultivation, and always on the same medium. The shorter the period of cultivation probably the better. If primary cultures could always be used for comparison, I believe that strains with intermediate characters of growth would to a large extent disappear, and each group would contract and become more sharply distinct from the other. It is, of course, too much to ask that cultural characters on such media as glycerin-broth-agar or -potato should be determined in primary cultures, but I am of opinion that early cultures should be exclusively used, that comparison should be made between cultures whose total duration of cultivation is approximately the same. If this were done I admit there would still be a considerable difference between the members of each group, but I believe that the two groups would show themselves to be distinct.

(9) It has been said that cultural characters and virulence go to some extent hand in hand. In the modification experiments, whenever alteration of virulence occurred, there was a corresponding alteration of cultural characters. The original cultures of low virulence grew well on glycerin media, and after they had acquired virulence for the calf, etc. they grew very badly on these media. But on the other hand an alteration of cultural characters may take place without any change of virulence, and virulent strains which would not at first grow on glycerin media were found to have lost no virulence after they had adapted themselves to this medium. Moreover, while the virulent strains originally differed greatly in their capacity to grow on glycerin media no corresponding difference of virulence was observed.

It may, however, be said that the two groups into which human tubercle bacilli are divided by their cultural characters are in the main the same two groups into which they are separated by their comparative virulence for the ox.

After one had gained some experiene, one seldom, if ever, was unable to foretell the comparative virulence of a strain from a cursory study of the characters of its early cultures. It was often on these grounds alone that the decision rested whether or no calves should be injected with 10 mg. doses. Only once was this dose injected unnecessarily, and the mistake was afterwards found to have been due to the use of an unsatisfactory batch of glycerin-serum tubes.

The opinions expressed in the above summary are based not only on the impressions gained day by day and from notes taken at the time, but also on the comparative examination of large numbers of typical cultures on all sorts of media which have been preserved. Early impressions could be checked by the gradual growth of experience, and opinion based on cultures grown a year or more earlier could be corrected by comparing the actual cultures with those grown to-day. Each culture tube was marked by the number of generation, and the total duration of cultivation up to the time of sowing that tube could be ascertained by comparing its date with the date of isolation.

An actual comparison of the relative amount of growth on serum with and without glycerin of certain selected strain may be of interest, though the results are much spoilt by variations in the total duration of artificial cultivation among the strains used.

The method was as follows: When serum cultures were sown for the purpose of getting bacilli with which to inject animals, other cultures were sown on tubes of the same serum to which 5 per cent. of glycerin had been added. The tubes used were large ones with a constricted neck, and the amount of surface exposed by the culture medium in each was 7 in. by more than 1 in. Several tubes of each kind were sown, and if any of them grew less well than their fellows they were rejected. Thus only cultures which were considered satisfactory were measured. At the end of three weeks' incubation all the bacilli from each set of cultures were removed and emulsified by rubbing between ground glass plates and suspended in a known volume of salt solution.

The relative quantity of bacilli in the two suspensions was determined by taking samples of each and centrifuging in capillary tubes, known to be of uniform calibre by careful calibration.

The results are given in the following table.

The serum of adult bovine animals has consistently proved to be a better medium for growing tubercle bacilli than the serum of the calf. The addition of glycerin to the latter makes it a far better medium for the cultivation of bacilli belonging to Group II., but the serum of adult bovine animals is not improved to the same extent. It would seem that it already possesses something which calf serum lacks and which to some extent can be replaced by glycerin. As a consequence, adult serum does not bring out the difference caused by the presence or absence of glycerin in so marked a manner as does calf serum. In the experiments now under consideration calf serum was employed except when otherwise stated. With the serum of one particular cow (No. 9), which was used in several of the experiments, the difference between the amount of growth on serum and glycerin-serum was particularly small.

TABLE SHOWING THE RATIO OF THE QUANTITY OF GROWTH ON SERUM TO THAT OF GLYCERIN-SERUM.

(1) GROUP II.

Virus.	Strain.	Total duration of artificial cultivation.	Generation of culture.	Age of culture in days.	Ratio of amount of growth on :	
					Glycerin-serum.	Serum.
H 26. "K.M."	Human kidney	13 months	11th	21	16·0	1
H 45. "F.M."	Human bronchial gland	11 ,,	9th	21	4·5	1
H 9. "C.T."	Calf 201	10 ,,	6th	21	3·5	1
H 18. "T.T."	Calf 405	8½ ,,	6th	21	6·0	1
H 15. "I.W."	Calf 413	8 ,,	9th	21	4·4	1
H 11. "E.D."	Calf 309	8 ,,	11th	21	4·5	1
H 30. "E.M."	Human mesenteric and bronchial glands.	4½ ,,	4th	21	3·3	1¶
H 47. "S.B."	Human hip joint	4 ,,	5th	21	1·67	1†
H 51. "H.M."	Human lung	4 ,,	5th	21	6·3	1‡
H 50. "P.H."	,, ,,	3 ,,	5th	21	2·9	1†
H 44. "D.C."	Human cervical glands	4 ,,	8th	21	3·8	1
H 43. "F.F."	Human knee joint	2 ,,	4th	17	2·6	1
H 41. "A.S."	,, ,, ,,	2 ,,	3rd	21	5·4	1
H 46. "H.W."	,, ,, ,,	2 ,,	6th	21	2·4	1
H 48. "W.P."	Human lung	1½ ,,	3rd	21	1·2	1†

* After growing 5 generations on glycerin-serum. † Serum of Cow 9. ‡ Serum of Cow 259. ¶ Pig serum used.

TABLE SHOWING THE RATIO OF THE QUANTITY OF GROWTH ON SERUM TO THAT OF GLYCERIN-SERUM.

(2) GROUP I.

Virus.	Strain.	Total duration of artificial cultivation.	Generation of culture.	Age of culture in days.	Ratio of amount of growth on :	
					Glycerin-serum.	Serum.
H 7. "C.M."	Calf 5	2½ years	17th	21	1·3	1†
,, ,,	,, ,,	2⅚ ,,	21st	21	4·0	1*
H 14. "F.S."	Human mesenteric glands	2 ,,	19th	20	1·44	1
,, ,,	,, ,, ,,	2 ,,	19th	21	1·	1‡
H 19. "S.W."	Goat 11	1½ ,,	16th	21	1·36	1
H 20. "F.L."	Calf 213	17 months	15th	22	1·88	1
H 31. "L.F."	Human cervical glands	14 ,,	12th	21	1·9	1
H 28. "C.L."	Rabbit 102	12 ,,	13th	21	1·5	1
,, ,,	,, ,,	12 ,,	14th	31	1·6	1**
H 16. "J.H." ¶	Calf 337	12 ,,	11th	21	1·2	1†
,, ,,	Calf 317	10 ,,	10th	21	0·88	1
H 29. "M.F."	Human cervical glands	8 ,,	10th	22	1·13	1
H 17. "Sp.B." ¶	Calf 553	3½ ,,	4th	21	0·76	1
,, ,,	,, ,,	3½ ,,	4th	21	0	N

** After growing for 1 year on glycerin-serum. † Serum of Cow 9. ‡ Serum of Cow 259.
¶ After increase of virulence.

TABLE SHOWING THE RATIO OF THE QUANTITY OF GROWTH ON SERUM TO THAT OF GLYCERIN-SERUM.

(3) MIXTURE OF GROUPS I. AND II.

Virus.	Strain.	Total duration of artificial cultivation.	Generation of culture.	Age of culture in days.	Ratio of amount of growth on :	
					Glycerin-serum.	Serum.
H 8. "S.C." and H 10. "B.S." [1st Mixed Culture]	H 8. "S.C."; Human mesenteric glands. H 10. "B.S." Calf 103.	"S.C." 2½ yrs. "B.S." 1½ yrs.	19th 14th	21	5·6	1

REPORT ON THE CULTIVATION OF TUBERCLE BACILLI AT BLYTHWOOD.

By Mr. H. J. HUTCHENS (Assistant Investigator).

REPORT ON THE CULTIVATION OF TUBERCLE BACILLI AT BLYTHWOOD FARM.

From November, 1903, *to June*, 1905, *by H. J. Hutchens.*

The work here reported was undertaken at Blythwood Farm, while acting as assistant to Dr. Cobbett, with the object of determining the cultural characters of tubercle bacilli derived originally from various tuberculous lesions in the human subject.

At the outset, considerable difficulty was encountered in isolating bacilli in pure culture and in propagating them under conditions of artificial cultivation. The methods of isolation and of cultivation and the preparation of the media selected on which to grow the bacilli in vitro are therefore also described.

In a few instances cultures have been raised by sowing the human material on suitable culture media, but in the vast majority of cases the human material was inoculated into experimental animals, and the lesions subsequently developing in the latter were the immediate source of the cultures.

The different human lesions used for experiment are described as viruses; and cultures obtained either from these lesions or from lesions produced by them in experimental animals as strains of bacilli. The animals from which the different strains were immediately derived are stated in every case.

In all, 141 strains of bacilli derived originally from forty-seven different human sources have been isolated and examined.

THE MEDIA USED FOR CULTIVATION AND THEIR PREPARATION.

The media most commonly used were—

α. Blood serum.

β. Blood serum to which a solution of glycerin had been added.

γ. Glycerin agar.

δ. Glycerin broth.

Preparation of the media.

α. *Blood serum.*—Blood for the preparation of serum was obtained from various animals, viz.—

1. Bull, cow and calf.
2. Horse.
3. Pig.
4. Goat.
5. Dog.
6. Cat.
7. Rabbit.
8. Guinea pig.
9. Monkey.

Blood was collected in suitable sterile vessels and the serum drawn off by means of a sterile bulb and cannula, distributed at once in convenient quantities into sterile test tubes, which were then heated gradually in a sloped position to a temperature of about 75°C. in an atmosphere saturated with water vapour until the serum was so firmly set that it no longer quivered when the tubes were tapped on the thumb-nail. As soon as the tubes had cooled they were carefully and completely sealed with paraffin wax. The tubes were finally incubated for three or four days before being used, in case some error of technique had permitted the entrance of contaminating organisms into one or more tubes.

An iridescence was generally observed on the surface of the serum when it had cooled after coagulation. This did not appear to seriously interfere with the growth of the tubercle bacillus provided that other conditions of the medium were satisfactory. Further experience, however, showed that the formation of an iridescent film could be almost, if not entirely, obviated by raising the temperature very gradually to the point of coagulation, and removing the serum from the inspissator immediately it was firmly set, and before the temperature reached an unnecessarily high degree.

β. *Blood serum with the addition of glycerin.*—Blood serum with the addition of 1·25 per cent. and 5 per cent. of glycerin was very largely used in the work under review. This medium was prepared by adding to ordinary serum before coagulation, in the former case 25 per cent. of its volume of a 5 per cent. solution of glycerin in water, and in the latter case 10 per cent. of a 50 per cent. solution of glycerin in water—the glycerin solutions being, of course, previously sterilized. The medium was then coagulated, sealed and incubated as in the case of blood serum.

γ. *Glycerin Broth.*—The perfectly fresh muscles from the calf or adult bovine animal were finely minced, and tap water added in the proportion of one litre to one pound of meat; 0·5 per cent. sodium chloride added, stirred and allowed to stand overnight. Next morning it was boiled, then strained, and subsequently 2 per cent. Witte's peptone added. It was then boiled again, filtered, and 5 per cent. glycerin added, being finally sterilized in the ordinary manner. The reaction of a broth so prepared was found to be neutral or very faintly acid to litmus, and to phenol-phthalein acid, approximately to the extent of 20 c.c. normal acid per litre.

δ. *Glycerin Agar.*—Was prepared by adding 2 per cent. agar to the glycerin broth prepared as above.

It was usual, especially in the latter part of the work, to seal with paraffin wax all tubes, flasks, and bottles, in which media were stored, and also while they were in the incubator after sowing.

THE SELECTION OF THE MATERIAL FROM WHICH TO ISOLATE BACILLI IN PURE CULTURE.

α. *Human.*—Cultures were raised in several instances directly from human material by scraping the tuberculous tissue and sowing tubes with the material so obtained. Mesenteric and cervical glands have proved particularly useful for this purpose.

β. *Bovine.*—The bovine experimental animals at Blythwood were, in the great majority of cases, inoculated subcutaneously in the neck with material of human origin.

Almost invariably a tuberculous lymphatic gland has been used and generally the prescapular, prepectoral or long posterior mediastinal. Organs such as the lung, liver, spleen and kidney of the bovine, though not often used, have not given good results. Experience early showed that old calcareous and fibrous lesions were also to be avoided, because of the frequency with which cultures sown with such material failed.

γ. *Guinea Pig.*—Any structure affected with the disease was found to readily yield a culture. As a rule a caseous and softened gland, *e.g.* the inguinal, post manubrial, omental or hepatic, or the omentum or spleen or—when the inoculation was intra-peritoneal—a scraping from the peritoneum or the lung have been used. The kidney and liver have not yielded such good results on the relatively few occasions on which they have been utilised.

δ. *Rabbit.*—The omentum, lung, or rib-marrow. Most of the rabbits were inoculated intraperitoneally.

ι. *Monkey.*—A softened and caseous lymphatic gland has been the tissue from which cultures have been sown.

ξ. *Goat.*—The prescapular or long posterior mediastinal gland or the kidney.

η. *Rat.*—Lung and spleen have been used.

When the animal was but slightly affected it was found advisable, in order to prevent disappointment, to sow tubes from several different organs or tissues. This was also done when the lesions present were old and calcareous. Similarly, if only one gland in the bovine or goat was affected better results were obtained by sowing from different parts than all from the same part.

The Technique of raising Primary Cultures.

Various procedures have been adopted for sowing the culture media. Thus, in the case of a softened caseous gland the capsule was sterilised by heat, a small opening made and the pultaceous matter squeezed out drop by drop and smeared over the surface of the medium with a platinum loop.

When the peritoneum was selected, the surface was scraped with a blunt knife and the material so obtained rubbed over the surface of the culture medium.

The omentum was dealt with in a variety of ways; thus, a small piece was sometimes cut off, placed on the surface of the culture medium, and then rubbed over its surface; or, after cutting off a small piece, the fluid and small pieces of tissue obtained by crushing between ground glass plates were rubbed over the medium; or again, if caseous and softened points were present, these were cut into and the material picked out on the point of the knife and used for sowing the tubes.

From the spleen or lung or tuberculous gland which had not softened, it was usual to cut out a small piece, or scrape the tissue with a knife and then sow the tubes, or, having cut out a piece, to crush it between ground glass plates and distribute the crushed tissue with a platinum wire or, if fluid, to pick it up with a piece of glass tubing drawn out to a capillary diameter and spray it over the medium.

After well distributing the material over the surface of the medium the tube was incubated without further interference until growth appeared.

The Development of Primary Cultures.

It has very rarely happened that no growth of tubercle bacilli has occurred when the animal tissue was sown, by one of the methods described, on to the surface of any good serum whatever.

A. *Media used for raising Primary Cultures.*

Coagulated blood serum obtained from various animals with and without the addition of glycerin have been the media commonly used for the purpose of raising primary cultures. Three or four tubes of coagulated blood serum were always used and generally one or two tubes of glycerin serum were sown at the same time. Occasionally a tube of glucose serum or serum agar has also been used. Other media have not been regularly employed for raising primary cultures in these investigations.

B. *Comparison of Values of different Media for Primary Cultures.*

Of the various media used, serum has been the most useful medium upon which to sow primary cultures, for while all tubercle bacilli from whatever source obtained grew more or less readily on serum, bacilli were met with which did not grow at all, or only with great difficulty in primary culture on glycerin serum.

In those cases in which a tube of serum and a tube of glycerin serum were inoculated with material from the same source it was generally found that growth appeared on the serum before it appeared on the glycerin serum.

Glucose serum appeared to be equal to, and perhaps rather better than, serum. And of the various kinds of serum which have been used the most successful results have been obtained with serum from the dog and pig; nevertheless the majority of my cultures have been raised in the first instance on bovine serum, and in my experience a rich golden serum from a milking Jersey cow is as good as any serum obtained.

Glycerin agar has not in my experience proved suitable for raising primary cultures. Serum agar, moreover, has only rarely been satisfactory.

C. *Characters of Growth in Primary Culture.*

These were very variable and were found to depend, in part upon the manner in which the material was sown, and in part upon the number of bacilli present which subsequently multiplied. Other factors influencing the characters of growth in primary culture were the media employed and in the case of serum media the quality of the medium.

When a piece of tissue was merely placed on the medium the bacilli multiplied on the tissue itself and the first evidence that multiplication had occurred was a slight rounding off of the minute ridges and projections and a generally somewhat moist and shiny appearance of the surface of the tissue. Later, minute rounded elevations appeared, and these often formed ultimately large heaped-up cream-coloured nodules or large "crusts."

On the other hand when the surface had been merely rubbed over with scrapings from the tuberculous tissue, or with tissue juices obtained by crushing the material between ground glass plates, or softened pultaceous matter had been sown in a thin layer, or an emulsion sprayed over the surface, the resulting growth had all the characters described later as occurring when subcultures were made on the same media; the only difference observed being that, as a rule, the growth in primary culture was scantier and made its appearance later than in subculture, consequently isolated discrete colonies which often attained considerable size were more commonly seen in the former than in the latter.

A difference between the growth on serum and on glycerin serum was often very well brought out in primary cultures. It was this, when the number of colonies was so small that each remained discrete from its neighbours the number developing on the glycerin serum was in comparable tubes (*i.e.*, tubes prepared from the same batch of serum and inoculated with similar material) often much smaller than on serum and the individual colonies much larger and whiter. This, however, in the case of primary cultures, was found to be a rule which has many exceptions.

In the few cases in which glucose serum has been used it has been noticed, on the other hand, that the number of colonies was very much greater and individuals correspondingly smaller than on serum. Hence, it sometimes happened that a thin ground glass layer formed on the glucose serum while on serum the colonies were discrete but fairly large in number and small in size.

The length of time which elapsed after the tissue had been sown and before growth became apparent to the naked eye varied within wide limits. Sometimes as early as the seventh or eighth day after sowing, frequently at the end of 10 days and very rarely after 3 or 4 days some evidence of growth was plainly visible. In a few cases it has been three or four weeks before the growth had progressed sufficiently far to be visible to the naked eye. In the majority of cases, however, growth made its appearance between the end of the first and the end of the second week.

When pieces of tissue had been sown, whether crushed or not, an early growth not infrequently escaped notice when examined with the naked eye until some experience had been gained in examining primary cultures microscopically. If a microscopical examination be made it could generally be determined at a much earlier period than was possible with the naked eye whether or no growth was taking place. Thus, it was quite usual to find on examining a cover glass preparation that considerable multiplication had occurred even after four days, and by making subcultures at this early period growth was frequently much accelerated. The microscopic appearance which was taken as evidence of growth was the presence of bacilli in clumps instead of occurring singly or in pairs or in relatively quite small clumps as they usually do in tissues.

In some cases with any of the media employed, and particularly in the case of horse serum, the appearance of visible growth was delayed until the third and fourth week. Meanwhile the surface of the medium became covered with a scum. As growth became apparent minute circular areas were seen from which the scum had disappeared. In the centre of each of these areas a minute, practically colourless, speck was found to be present; this was a centre of commencing growth which progressed and assumed the ordinary characters of growth on that medium.

Microscopically the bacilli obtained from primary cultures on serum and glycerine serum were very noticeably shorter and more uniformly stained than those in the animal tissues with which the tubes were inoculated.

D. *Contaminations.*

When sowing primary cultures it was found advisable to inoculate several tubes, and these from different organs or glands, in order to minimise the risk of failing to obtaining a culture. As experience was gained in technique the number of contaminated tubes was considerably reduced, but however much care was bestowed in sowing primary cultures from even recently killed animals my experience showed that sometimes contaminating organisms were occasionally present in the animal tissue : *e.g.*, culture tubes sown with material from the bovine lung have repeatedly grown organisms other than tubercle ; on the other hand the lung of the rabbit has proved one of the most satisfactory tissues with which to sow cultures.

Contaminations generally appeared within forty-eight hours, and were, as a rule, sufficiently obvious ; if, however, there was some doubt as to the purity of a culture the condition of the fluid at the bottom of the tube will often assist in settling the difficulty ; if the fluid be milky the tube is impure.

But while the majority of contaminations were apparent within forty-eight hours, organisms were sometimes present which were as slow or even slower in producing a recognisable growth than tubercle, and in such a case it has happened that the growth had all the appearances of tubercle, the fluid at the bottom of the tube was clear and every indication of contamination was absent. The only certain method therefore of determining whether a culture was pure or not was to examine it microscopically.

When dealing with the original human material contaminating organisms have occasionally been present which were of such a nature as to be unable to grow under the ordinary conditions of tubercle cultivation, and consequently pure cultures of tubercle bacilli were sometimes raised from material which microscopically was very unpromising.

Pure cultures of tubercle bacilli have however occasionally been recovered from contaminated tubes. The ordinary method of obtaining pure culture by the method of dilution not being applicable, the best chance was found to lie in picking off a trace of growth from various parts of the serum, examining under the microscope, and, if no foreign organisms were present, to sow that particular piece on to a serum tube. When the colonies were discrete then of course recovery was more easily effected.

SUBCULTURES.

When subcultures were made merely for the purpose of perpetuating any strain of bacilli, it was found to be desirable that they should be made from serum on to serum and at intervals of a month to six weeks. The importance of making subcultures from serum on to serum lies in the fact that, as these experiments proved, whatever medium was used the bacilli slowly underwent change in their cultural characters, and this change has been found to be least marked, and to occur most gradually, when serum is used. It has often happened that subcultures have been made from cultures which were much older than six weeks, but experience showed that a risk was then incurred that the bacilli might have died, and this especially if the serum upon which they were last sown had been a poor one.

But when the object of making subcultures was to determine the cultural characters of a particular strain then various other media were used, and those relied upon for this purpose have been :—

Serum.
Glycerin serum.
Glycerin agar.
Glycerin broth.

And as the result of my experience I have come to the conclusion that in order to get reliable results with these "differentiating media" it is absolutely necessary that they should be sown from young vigorous cultures, seven to fourteen days old, growing on a good serum, and as soon after isolation from the animal tissues as possible. And the method I came to adopt as the most desirable when attempting to differentiate strains was to sow three or four serum tubes from the primary culture on serum, and from these to inoculate two or three tubes of serum, glycerin serum, and glycerin agar—if only one tube of each was sown it was found that error was very liable to creep in and give a false impression.

GENERAL STATEMENT OF THE APPEARANCE OF GROWTH OF TUBERCLE BACILLI ON EACH OF THE FOUR MEDIA—SERUM, GLYCERIN SERUM, GLYCERIN AGAR, AND GLYCERIN BROTH.

Even with the same strain of bacilli results were very variable, and depended, in addition to the readiness or reluctance of the particular strain under investigation to grow on artificial media, upon the age of the culture from which subcultures were made, the quality of the medium upon which the bacillus was growing, and of that upon which it was sown, the amount of material sown, and the length of time it had been under artificial cultivation. Reliable comparisons, therefore, between different strains were only possible when these disturbing factors had, as far as possible, been excluded.

Serum.—When a good serum has been used as the culture medium no difficulty has been experienced in obtaining a growth with any of the strains of bacilli examined ; but at the same time differences in the rate of growth and in the degree of luxuriance ultimately attained have been constantly observed. Further, it has been found that those strains which in general grew with the greatest luxuriance and most rapidly on serum were those which grew also most readily and rapidly and with the greatest luxuriance on other media ; and conversely, those whose yield on serum was sparse were those which grew sparingly and slowly and often with difficulty on other media. But though this has been found to be true as a general observation, the differences in the growth on serum were not so constant nor so well marked as the differences found to exist in the growth on glycerin serum, glycerin agar and glycerin broth.

When every precaution had been taken to eliminate as far as possible every disturbing factor it was found that while in some cases growth took place relatively somewhat slowly and the surface became covered eventually with a thin, colourless, almost invisible, dull, uniform, ground-glass layer, in other cases multiplication took place distinctly more rapidly, so that within a week the surface of the medium was covered with a thick layer of growth generally more or less pigmented (if the medium were pigmented) with rounded hemispherical elevations projecting from the general surface of the growth. In these cases, after further incubation the layer of growth had thickened considerably, become more pigmented, the elevations had increased in size and number, and the growth had a tendency, often considerable, to extend on to the glass of the tube, and occasionally a certain degree of wrinkling of the layer was noticed.

These were the two extreme types of growth, but were not by any means the only ones obtained. Every possible intermediate type was met with, filling up the gap between those described, that is, between the least luxuriant, and the most luxuriant. And in all cases the differences were differences in degree only.

When the growth from a serum tube, which often sticks very tightly, was washed off or gently lifted off with a platinum spatula it was found that the surface of the serum was dotted with innumerable minute holes representing points where the bacillus in its growth had actually extended into the surface layer of the medium.

Glycerin Serum.—The appearances resulting from growth on this medium were essentially similar to those already described as seen on serum. The differences in the rate of growth and in the degree of luxuriance attained were, however, more marked than on serum, for while no difficulty was experienced in obtaining a growth on serum with any of the strains, some, those, namely, which exhibited the least luxuriance on serum, grew on glycerin serum only with difficulty and after a delay which was often considerable. On the other hand, those bacilli which grew the most readily and most luxuriantly on serum produced in the same space of time a growth which was in every respect equal to that obtained on serum, and in some cases, especially when the serum was not of the best, the growth on glycerin serum excelled that on serum.

In many cases the growth on glycerin serum was slower in making its appearance than on serum, and in practically every case the growth is whiter on the former than on the latter (when a pigmented serum was used). The layer of growth, too, was frequently more wrinkled in the case of the more luxuriantly growing bacilli when grown on glycerin serum than when grown on serum without the addition of glycerin.

On removing the layer of growth the surface of the medium was frequently seen to be pitted as in the case of serum.

On Glycerin Agar.—In some cases no growth at all has occurred for some weeks, and when the maximum has been finally attained it is very scanty. In such cases the surface did not become uniformly covered, but growth consisted merely of isolated colonies often very few in number, sometimes large, sometimes small, but in either case generally surrounded by a spreading, often delicately wrinkled, almost colourless halo. These colonies often became considerably heaped up, ridged on the surface and hollowed at the base. If left for three or four months the growth occasionally took a rosette form.

Some strains developed after some delay a very thin, almost colourless layer of growth which, at first uniform, later became irregularly and sparsely studded with small cream-coloured hemispherical nodules.

With other strains again growth commenced without delay, progressed rapidly, and the ultimate yield was considerable, consisting of a thick cream-coloured generally wrinkled layer which was thickly studded with projections from the general surface, these projections resembling "miniature mountain ranges." Moreover, these strains of bacilli had a great tendency to grow beyond the surface of the agar on to the glass sides of the tube.

Intermediate appearances of almost infinite variety have been obtained on this medium.

On Glycerin Broth.—Various methods have been adopted for sowing broth cultures. In most cases the film on the fluid at the bottom of a serum tube has been lifted off with a many-looped platinum wire and floated on to broth. The best results were obtained by transferring this film as soon as it had formed, and in some cases, in order to increase its extent before transferring to broth, a few drops of broth have been added to the fluid already in the serum tube, and the culture replaced in the incubator for a week or so.

In a few cases the film so transferred has at once begun to extend, and has covered the surface of a 6-oz. bottle, laid on its broader face, in two or three weeks or even earlier. But in a majority of cases no extension, or at most a very slight extension, of the film has occurred for some time, perhaps three or four weeks. During this period the pellicle, if the bacilli were alive, thickened and developed very small, white, scattered hemispherical points, and sometimes downgrowths into the depths of the fluid took place. But in these cases once the film had started to extend its area growth over the surface of the liquid medium was generally very rapid, so that the surface was covered not infrequently in so short a space of time as fourteen days. In some cases it has been three or even four months after transferring the film before any increase in area has been observed. It has, however, been frequently noticed that growth ceased before the whole surface was covered, and this has been the case with all strains, whatever their origin.

The resulting pellicle was in some cases very thin and delicate, practically colourless, flat, uniform, and readily broken up into small islands, with but small tendency to climb the sides of the bottle and floating with difficulty.

In other cases the film was somewhat thicker, greyish-white in colour, and generally reticulated.

With many strains of bacilli the pellicle was relatively thick, cream-coloured and opaque, floating much more readily than the thinner films, often thrown into delicate folds and showing a great tendency to climb the sides of the bottles, sometimes to such an extent that the roof was half occupied by the extension of the film. In such cultures small white nodular projections were often seen.

Downgrowths from the under surface of the original material sown occurred indiscriminately, whatever the nature of the resulting pellicle. These stalactites were particularly well marked when there had been much delay before growth began to extend laterally.

The growth on broth has been found to be very capricious, and with the same strain broth has given more variable results than any of the other media described.

COMPARISON OF VALUES OF EACH OF THESE MEDIA FOR COMPARATIVE PURPOSES.

While the various strains of tubercle bacilli investigated grew on serum with varying degrees of luxuriance, the medium was found to be of relatively small value for comparative purposes on account of the slight differences which have been found to exist between the most luxuriantly growing strains and those which grow most scantily.

But the differences observed between the various strains on glycerin serum, glycerin agar, and glycerin broth, have been in each case much more marked than on serum.

COMPARISON OF GROWTHS ON SERUM AND ON GLYCERIN SERUM.

In these comparisons the same batch of serum has been used in the whole serum and in the glycerin serum tubes.

In comparing the growths produced by the various strains on these two media, it has been found that when a strain was such as to grow only poorly on serum, then its growth on glycerin serum would also be scanty, and in some cases did not occur at all in early sub-culture, and on the other hand, a bacillus which grew luxuriantly on serum grew luxuriantly also on glycerin serum. It has further been observed that in the case of the more luxuriantly growing bacilli, when a poor sample of serum was used, the growth on glycerin serum exceeded in amount the growth on serum, making it appear as though glycerin acted as a substitute for, or supplemented, certain nutritive substances which are absent from or deficient in a poor serum.

With the best samples of serum this difference in the relative amounts of growth of serum and glycerin serum did not occur.

DIFFERENTIATION OF STRAINS AND VIRUSES.

The characters of growth of tubercle bacilli on serum, glycerin serum, glycerin agar, and glycerin broth have already been considered. Very great difficulty has been experienced in obtaining constant results as regards these characters, but by multiplying, repeating, and controlling the experiments, and by taking every possible care to eliminate as far as possible any disturbance due to factors under control, it has been found that well-marked differences exist between the various strains of different viruses and in a few cases between the different strains of the same viruses.

Some strains of bacilli when sown in primary culture have grown readily on both serum and glycerin serum, and in a relatively short space of time have produced abundant growths on both these media. And in some cases it has been noticed that the amount of growth ultimately resulting on the glycerin serum medium is even more luxuriant than that on the serum medium. Such strains in sub-culture have grown easily, readily, and luxuriantly on serum, glycerin serum, glycerin agar, and glycerin broth. That is to say, that when sown on these media from an early and young subculture growth commenced early, proceeded rapidly, attained its maximum soon and was finally very copious.

Other strains grew well on serum and on glycerin serum both in primary and in subculture, but not quite so rapidly nor so luxuriantly as those already described; and, further, it has been found that these strains when subcultured on to glycerin agar and glycerin broth only commenced to grow after some delay, and it has not infrequently happened that growth was only obtainable on these two last-named media when the strain had been in culture for some weeks, and had passed through several subcultures on serum or glycerin serum. Even when growth was finally obtained, it did not attain the luxuriance of those strains above described.

In the case of some other bacilli, growth has very rarely indeed been obtained on glycerin serum in primary culture, and then traces only, and that after a long period of delay. Such bacilli have been found to grow far better on serum than on any of the other three media under consideration. But even on serum the growth was relatively slow and continued for a long time, while the amount ultimately obtained was small. On glycerin serum it frequently happened that no growth was obtained in the first two or three sub-cultures, and even when it did occur was scanty and very slow in appearing. On glycerin agar it was very rarely indeed that the smallest trace of growth was obtained until these strains had been in culture for some months, and even then it was generally many weeks at the earliest after sowing on the medium before indications of multiplication were visible, and, further, with these bacilli, growth was, as a rule, limited to one or two hemispherical colonies, or a few very small colourless points of growth. On glycerin broth growth was as difficult to obtain as on glycerin agar. When first isolated from the living tissues, bacilli of this character have in my experience consistently failed to grow on glycerin broth, and it was only after repeated sub-culture on serum that a film was ultimately obtained, and even then the film was very thin and delicate.

My experiments have shown that after long-continued cultivation outside the body the growth characters undergo a slow but quite definite change. The longer a strain of bacilli has been kept in artificial culture the more readily and luxuriantly has it grown, and strains which when first isolated were difficult to grow, and on certain media did not grow at all, became so changed as to grow comparatively easily and well on any of the media mentioned.

Conclusions.

From the examination of a very large number of strains of tubercle bacilli derived from many human sources which is summarised in the foregoing report, the following results have been obtained;—

1. In staining properties and microscopic appearances no essential differences have been found in culture between the various strains examined.
2. In cultural characters the only differences which were made out were differences in amount of growth and in time of growth. In all other respects all the strains examined agreed in their cultural characters.
3. The relative capacity of the several strains to grow in artificial culture when first isolated from the tissues of the body varied within limits, for while some grew relatively slowly and scantily, and on some media with great reluctance, others there were which grew more rapidly, more luxuriantly, and with greater readiness, and others, again, with even greater relative rapidity, luxuriance, and ease.
4. The relative capacity to grow outside the body was, however, not constant, for, on the one hand, when any strain of bacilli was kept in artificial culture for some time it was relatively more eugonic than it was at first, and this was equally true of all strains, whatever their initial gonic power. It has also been noticed that bacilli after passage through a rabbit or a goat are slightly more eugonic than after passage through a bovine animal or a guinea-pig. And, on the other hand, in three cases a eugonic bacillus after passage through a series of bovine animals has been converted into a dysgonic bacillus, and cultures obtained from intermediate animals of the passage series was less eugonic than the former and more eugonic than the latter. It has also been found possible to alter gonic power by altering the character of the culture medium. All strains of bacilli grew more rapidly, luxuriantly, and easily on a rich than on a poor serum, and on one that was moist than on one which was dry. Again, for instance, in the case of broth cultures, concentration, dilution, or addition of acid all changed the relative eugony or dysgony of any strain of bacilli.
5. The cultural characters of many of the strains which I have recorded in this report corresponded in every respect to the German "Typus humanus"; others, again, were identical in every detail with their so-called "Typus bovinus." Filling up the gap between the typical typus humanus and the typical typus bovinus a large number of strains of bacilli have come under my observation, which exhibit characters intermediate between the two.

Table showing the different viruses arranged according to the source of the human material and the number of strains of each virus examined.

				No. of strains examined.
Axillary glands:—				
	H 33.	**R.T.**		1
Bone and gland from a case of multiple osteitis:—				
	H 15.	**I.W.**		2
Bronchial glands:—				
	H 13.	**A.D.**		3
	H 21.	**G.B.**		2
	H 32.	**Y.W.**		1
	H 35.	**C.B.**		1
	H 45.	**F.M.**		1
	H 22.	**F.W.**		1
Cervical glands:—				
	H 28.	**C.L.**		8
	H 29.	**M.F.**		5
	H 31.	**L.F.**		1
	H 38.	**J.M.**		1
	H 39.	**M.B.**		1
	H 44.	**D.C.**		1
	H 27.	**B.D.**		1
	H 34.	**C.U.**		1
Joints:—				
	H 16.	**J.H.**	*knee*	10
	H 9.	**C.T.**	*wrist*	3
	H 43.	**F.F.**	*knee*	1
	H 41.	**A.S.**	,,	2
	H 46.	**H.W.**	,,	1
	H 47.	**S.B.**	*hip*	1
	H 42.	**M.R.**	*ankle*	1
	H 11.	**E.D.**	*elbow*	2
Kidney:—				
	H 26.	**K.M.**		2
Lung:—				
	H 36.	**M.D.**		1
	H 23.	**J.P.**		4
	H 50.	**P.H.**		1
	H 48.	**W.P.**		2
	H 25.	**A.T.**		2
	H 22.	**F.W.**		3
Lupus Vulgaris:—				
	H 53.	**D.H.**		1
Mesenteric glands:—				
	H 10.	**B.S.**		10
	H 14.	**F.S.**		11
	H 7.	**C.M.**		4
	H 20.	**F.L.**		6
	H 32.	**Y.W.**		3
	H 19.	**S.W.**		4
	H 49.	**T.C.**		1
	H 18.	**T.T.**		4
	H 8.	**S.C.**		5
	H 36.	**M.D.**		1
	H 12.	**H.N.**		5
Mesenteric and bronchial glands:—				
	H 30.	**E.M.**		2
Sputum:—				
	H 17.	**Sp. B.**		7
	H 2.	**Sp. A.**		9
Testis:—				
	H 40.	**J.G.**		1

DETAILED EXAMINATION OF VIRUSES.

* Cultures originally isolated by Dr. Cobbett.
† Cultures originally isolated in conjunction with Dr. Cobbett.

H 2. Sp. A.

Source of the Human Material.—Mixed Sputum.

The cultures examined were obtained from :—

* G.P. 340 inoculated with an emulsion of the Mesenteric and Hepatic Glands of Heifer 11	2.iv.03
* G.P. 434 „ „ „ various Glands of Calf 79	6.iv.03
* G.P. 534 „ „ „ the Posterior Mediastinal Gland of Calf 93	14.v.03
* G.P. 618 „ „ „ „ Prepectoral Gland of Calf 83	12.viii.03
* The Local Lesion of Calf 163	14.viii.03
* G.P. 651 inoculated with emulsion of tissues of Calf 91	24.viii.03
† G.P. 690 „ „ „ „ Calf 111	9.x.03
† G.P. 664 „ „ „ „ Calf 153	10.x.03
† G.P. 777 „ „ „ „ Pig 3	12.x.03

Characters of growth in Sub-culture.

In all cases the same, viz.:—

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
All H 2. Sp. A.	Very good	Very good	Fairly easily growing and moderate yield	Fairly easily obtained and moderately thick.

H 7. C.M.

Mesenteric Glands of a child aged 3 years who died of tuberculous meningitis.

Source of the Cultures.	Sown.
*G.P. 448 from an emulsion of Calf 5	1.iv.03.
*G.P. 552 from supra-mammary gland of Cow 73	12.vi.03.
*G.P. 646 from anterior mediastinal gland of Calf 103	9.x.03.
*G.P. 708 from prescapular gland of Calf 115	12.x.03.

Characters of growth in Sub-culture.

In all cases the same, viz. :

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good ; fairly rapid.	As on serum.	Poor: much delayed and increase slow.	Moderate yield ; growth obtained fairly easily after being in culture some time.

H 8. S.C.

Mesenteric Glands of a child aged 11 months.

Source of Cultures.	Sown.
The Human Material—	
Calf 305	16.i.04
Prescapular Gland of Calf 303	13.iv.04
Thoracic Gland of Calf 361	30.v.04
G.P. 1230 from Local Lesion of Calf 275	28.vi.04

Characters of Growth in Primary Culture.

On serum—luxuriant.
On glycerin serum—luxuriant.

H 8. S.C.—*contd.*

Characters of growth in Sub-cultures.

In all cases the same, viz:—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Very good.	Very good: thick, white and opaque.

H 9. C.T.

Material removed by operation from a tuberculous wrist of a Man aged 55 years.

Source of Cultures.	Sown.
G. P. 981 from Calf 183 (prescapular gland)	12.i.04.
G. P. 996 from Calf 183 (prescapular gland)	9.ii.04.
G. P. 1081 from Calf 201 (prescapular and prepectoral glands)	19.v.04.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.	Dextrose Serum.
Calf 183 G. P. 981	Good.	Very good.	—
Calf 183 G. P. 996	Good.	—	—
Calf 201 G. P. 1081.	Good.	Very good.	Good.

H 10. B.S.

Mesenteric Glands from a child $1\frac{9}{12}$ *years.*

Source of Cultures.	Sown.
†G.P. 725 from Calf 35 (prescapular gland)	8.x.03.
†G.P. 1, from G.P. 771, 744, from G.P. 661, from G.P. 593, from G.P. 386, from original material	9.x.03.
G.P. 757 from Calf 113 (prescapular gland)	31.x.03.
G.P. 798 from Goat 3 (prescapular gland)	9.xi.03.
G.P. 791 from Calf 191 (prescapular gland)	11.xi.03.
Heifer 231 (prescapular gland)	16.xii.03.
G.P. 952 from Cow 295 (milk)	21.iii.04.
Monkey 59 (cervical gland)	16.vii.04.
Calf 387 (liver)	27.vii.04.
Heifer 249 (prepectoral gland)	16.viii.04.

Characters of growth in Primary Culture.

On serum.—The number of colonies developing was generally small and individuals remained discrete. They were nearly colourless, minute and hardly raised above the surface.

On glycerin serum.—Rarely any growth and then only after a long lapse of time, and the yield ultimately very poor.

H 10. B.S.—*contd.*

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Scanty, thin, colourless, and relatively slow-growing.	Very poor, growth much delayed and often not obtained in early sub-cultures. Multiplication much slower than on serum.	Cultures only obtained after being in artificial culture some time. Very slow-growing and ultimate yield scanty.	Not obtained until after being in culture some time. Film very thin, uniform and colourless. * See footnote.

The cultures obtained from the goat grew rather more easily and somewhat more copiously than the other cultures.

* Bacilli obtained from Heifer 231 after being in artificial culture for 15 months covered the surface of glycerin broth contained in a Winchester quart and laid on its side in 14 days.

H 11. E.D.

Synovial Membrane from the elbow joint of a female aged 10 years, removed by operation.

Source of the Cultures.	Sown.
G.P. 918 from prescapular gland of Calf 221 - - - - - -	23.xii.03
G.P. 1298 from portal lymphatic gland of Calf 309 - - -	23.vii.04.

Characters of growth in Primary Culture.

On serum - - - - - Good
On glycerin serum - - - Fair in 33 days

Characters of growth in Sub-culture.

In both cases the same, viz. :—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Copious.	Thick, white, easily grown and much folded.

H 12. H.N.

Messenteric Glands from a male aged 15 years.

Source of Cultures.	Sown.
*G.P. 577 from original material - - - - - - - - -	21.vi.03
*Calf 123 - - - - - - - - - - - - - -	27.viii.03
G.P. 988 from G.P. 928–930 (prescapular and prepectoral gland of Calf 189) -	13.i.04
The thoracic gland of Calf 319 - - - - - - - - -	10.iii.04
Calf 363 - - - - - - - - - - - - - -	22.vi.04

Characters of growth in Primary Cultures.

Source.	Serum.	Glycerin Serum.	Serum Agar.
Calf 123 G.P. 988 - - -	—	—	Luxuriant.
Calf 319 - - - - -	Luxuriant.	—	—
Calf 363 - - - - -	Very good.	Very good.	—

Characters of growth in Sub-culture.

In all cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Good, sometimes wrinkled.	Fairly thick, opaque film.

H 13. A.D.

Bronchial Glands and Spleen of a child aged 4 years. Disease probably primary in Bronchial Glands.

Source of the Cultures.	Sown.
Calf 301 (Thoracic Gland) - - - - - - -	20.i.04
Prepectoral Gland of Calf 321 - - - - - - -	23.iii.04.
Spleen of Rat 15 from Thoracic Gland of Calf 301 -	5.vii.04.

Characters of growth in Primary Culture.

Calf 301. On serum, good in 19 days.
„ 321. On serum, good.
Rat 15. {On serum, fair.
{On glycerin-serum, nil.

Characters of growth in Sub-culture.

These exhibited differences, viz. :—

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Calf 301	Very good.	Very good.	Very good; generally wrinkled.	Thick white film, often thrown into folds.
Calf 321.	Good: fairly rapid.	As on serum.	Very poor, much delayed and grows slowly.	Thin, colourless and transparent, and obtained with some difficulty.
Rat 15.	Scanty and relatively slow growing.	Very poor: growth delayed and increase slow.	Very scanty: a delay of several weeks occurs before growth begins and then proceeds slowly.	Scanty, very thin, transparent and delicate.

H 14. F.S.

Mesenteric Glands from a Child aged 4½ years.

Source of Cultures.	Sown.
Prescapular gland of Calf 125 - - - - - - - -	1.vii.03.
G.P. 587 from original material - - - - - - -	24.vii.03.
G.P. 769 from Heifer 197 (prescapular gland) - - - - -	15.x.03.
Mesenteric gland of Calf 181 - - - - - - - -	24.x.03.
G.P. 894 from Calf 205 (prescapular gland) - - - - -	19.xii.03.
G.P. 838 from Heifer 243 (prescapular gland) - - - - -	21.xii.03.
G.P. 1103 from Cow 235 (uterus) - - - - - - -	18.iii.04
G.P. 1108 from Calf 327 (hepatic gland) - - - - - -	22.iii.04.
G.P. 1104 from Cow 235 (uterus) - - - - - - -	2.iv.04.
G.P. 1109 from Calf 327 (hepatic gland) - - - - - -	12.iv.04.
G.P. 1106 from Calf 327 (liver)- - - - - - - -	30.v.04.

H 14. F.S.—*contd.*

Characters of growth in Primary Culture.

Calf 205	G.P. 894	On serum. Thin, colourless and slow growing. On glycerin serum. In two cases, one or two very small colourless colonies developed after the lapse of a long period of time.
Heifer 243	G.P. 838	
Cow 235	G.P. 1103	
Calf 327	G.P. 1108	
Cow 235	G.P. 1104	
Calf 327	G.P. 1109	
Calf 327	G.P. 1106	

Characters of growth in Sub-cultures.
In all cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Thin, colourless, scanty and relatively slow growing.	Often not occurring in early sub-cultures when obtained only after some lapse of time. Ultimate yield very poor.	Growth very long delayed and very sparse. Only obtained after being in culture some time.	Growth only occurred after being in artificial culture some time and the film then obtained was very thin and colourless.

H 15. I.W.

Fragments of Bone and an Enlarged Gland removed by operation from a case of Multiple Osteitis occurring in a child aged 4 *years.*

Source of Cultures.	Sown.
(2) Prescapular and prepectoral glands of Calf 413 -	4.viii.04.
(1) Calf 311 (prescapular gland) - - - - - -	9.v.04.

Characters of growth in Primary Culture.
On serum a luxuriant growth.

Characters of growth in Sub-culture.
In both cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Very good and rapidly growing. No delay in appearance of growth.	Easily obtained. Extension rapid and film of moderate thickness.

H 16. J.H.

Synovial Membrane from the knee joint of a man aged 38 *years removed by operation.*

Source of the Cultures.	Sown.
*G.P. 609 from human material - - - - - -	12.viii.03
*Calf 157 - - - - - - - - - - - -	24.viii.03
G.P. 872 from local lesion of Calf 187 - - - - - -	23.i.04
Prescapular gland of Calf 273 - - - - - - - - -	10.ii.04
Local lesion of Calf 355 - - - - - - - - -	24.iii.04
Calf 337 - - - - - - - - - - - -	30.iv.04
G.P. 1141 from prescapular gland of Calf 355 - - - - -	19.v.04
Calf 317 - - - - - - - - - - - -	13.vi.04
Prepectoral gland of Bull 423A - - - - - - -	30.vii.04
Bronchial gland of Calf 559 - - - - - - - -	12.xii.04

H 16. J.H.—*contd.*

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.	Dextrose Serum.
Calf 187 G.P. 872.	Good.	Some growth.	—
„ 273.	Fair growth.	Very good.	–
„ 355	Good.	Good.	-
„ 337.	Very good.	Nil.	Good.
„ 355 G.P. 1141.	Very good.	Some growth.	
„ 317.	—	—	Good.
Bull 423A.	Good in 18 days.	—	—
Calf 559.	Very good.	—	—

Characters of growth in Sub-culture.

These exhibited differences which are set out below.

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
G.P. 609 Calf 157 Calf 187, G.P. 872 Calf 273	Very luxuriant.	Very luxuriant.	Very good; thick, nodular, and wrinkled.	Very good, thick, opaque and folded.
Calf 355 Calf 337 Calf 355, G.P. 1141 Calf 317	Good, fairly rapid.	Good.	Poor, delayed and increase slow.	Moderate yield obtained with some difficulty.
Bull 423A Calf 559	Scanty, relatively slow-growing.	Scanty and slow-growing. Appearance of growth generally much delayed in early sub-culture.	Very scanty and very slow-growing. Growth not obtained in early sub-cultures, and when obtained only after a delay of some weeks.	Very scanty, thin and colourless. Growth obtained only after being in culture some little time.

H 17, Sp, B,

Mixed Sputum.

Source of the Cultures.	Sown.
Prescapular gland of Calf 265	11.ii.04
„ „ Calf 391	25.v.04
G.P. 83 from Calf 277 (prescapular gland)	27.iv.04
G.P. 235 from Calf 339 (prescapular and prepectoral glands)	8.vii.04
Prepectoral gland of Calf 555	19.i.05
Thoracic gland of Calf 555	19.i.05
Long thoracic gland of Calf 531	20.i.05

H 17. Sp. B.—*contd.*

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.
Calf 265	—	Luxuriant. Large varied white hemispherical colonies.
„ 391	Copious.	Copious.
„ 277 G.P. 81	—	Good.
„ 339 G.P. 235	Very scanty.	
„ 553	Fair growth. 6 weeks.	Nil. 6 weeks.
„ 555	Fair growth.	— —
„ 531	Scanty.	——

Characters of growth in Sub-culture.

These exhibited differences which are set out below :—

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Calf 265	Very luxuriant.	Very luxuriant.	Very good.	Easily obtained, thick film and often folded.
„ 277				
„ 391				
„ 339				
Calf 555	Scanty and relatively slow growing.	Very scanty and slow-growing, appearance of growth delayed.	Very scanty and slow-growing. Delay of several weeks before growth commences.	Scanty and slow-growing. Not obtained in early subcultures.
„ 531				

H 18. T.T.

Mesenteric Gland from a child $2\frac{5}{12}$ *years.*

Source of Cultures.	Sown.
G.P. 999 from Calf 131 prescapular gland after passage through G.Ps.	1. ii. 04.
G.P. 1174 „ „ „ „ „ „	20. iv. 04.
G.P. 1173 „ „ „ „ „ „	27. iv. 04.
Calf 405 „ „ „ „ „ „	21. vi. 04.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.
Calf 131 G.P. 999	—	Good.
G.P. 174	Good.	—
G.P. 173	Good.	—
Calf 405	—	—

Characters of growth in Sub-culture.

In all cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Good and only slightly delayed.	Fairly thick film.

H 19. S.W.

Mesenteric Glands from a child $2\frac{9}{12}$ years old.

Source of Cultures.	Sown.
Prescapular gland of Heifer 239 - - - - - -	3.iii.04.
Thoracic gland of Goat 11 - - - - - -	4.iii.04.
G.P. 1118 from Heifer 239 (milk) - - - - -	22.iii.04.
G.P. 1119 „ „ „ - - - - - - -	23.iv.04.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.
Heifer 239 - - - - -	Very good. 21 days.	Good. 21 days.
Goat 11 - - - - -	Copious. 28 days	
G.P. 1118 - - - - -	Very good.	
G.P. 1119 - - - - -	Copious.	Copious.

Characters of growth in Sub-culture.

In all cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good : fairly rapid growth.	Good : fairly rapid growth.	Much delayed : very slow growing, and ultimate yield very poor.	Difficult, and only obtained after being in culture some time. Film thick.

The cultures through the goat were rather more eugonic than those from the bovine or guinea-pig.

H 20. F.L.

Mesenteric Gland of a child aged $2\frac{10}{12}$ years.

Source of the Cultures.	Sown.
Prepectoral gland of Calf 213 - - - - - - - -	6.iv.04.
Prescapular gland of Calf 253 - - - - - - -	11.vii.04.
Rabbit 110 from the prescapular gland of Calf 253.	23.ix.04.
Rabbit 112 „ „ „ „ „	22.ix.04.
Lung of Rabbit 111 from prescapular gland of Calf 253 - - -	2.viii.04.
G.P. 307 from cerebro-spinal fluid of Calf 403 - - - -	9.viii.04.

Characters of growth in Primary Culture.

On serum a relatively good growth, though in some cases scanty.

On glycerin serum a good growth was sometimes obtained.

Characters of growth in Sub-culture.

In all cases the same, viz. :—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good.	Good.	Poor, generally delayed and increase slow.	Moderate yield, but obtained with great difficulty.

Note.—Cultures from the rabbits were relatively rather more eugonic than from the bovine

H 21. G.B.

Bronchial Gland of an adolescent aged 16 *years.*

Source of the Cultures.	Sown.
*Thoracic Gland of Calf 447 - - - - - -	28.ix.04.
G.P. 417 from Calf 447 (Prescapular Gland) - -	23.xi.04.

Characters of growth in Primary Culture.

Source.	Serum.	Serum Agar.
Calf 447 - - - - -	Scanty growth	—
Calf 447 G.P. 417 - -	Fair growth	Nil.

Characters of growth in Sub-culture.

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Calf 447 - -	Good fairly rapid	About equal to that on Serum	Scanty and delayed	Difficult to grow ; thin film.
Calf 447 G.P. 417	Good	Good	Fair yield and grew with but little delay	Moderately thick film.

H 22. F.W.

Lung and Bronchial Gland from a female aged 16 *years.*

Source of Cultures.	Sown.
*From the bronchial gland—	
Prescapular gland of Calf 293 - - - - - -	1.iii.04.
†From the Lung—	
Spleen of Calf 399 - - - - - - - -	7.vi.04.
G.P. 1200 from Calf 291 (prescapular gland) - - -	4.v.04.
Thoracic gland of Calf 457 - - - - - -	3.viii.04.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.	Dextrose Serum.
Calf 293 - - - - -	Good.	Very good.	—
Calf 399 - - - - -	—	—	Good.
Calf 291 G.P. 200 - - -	Good.	—	—
Calf 457 - - - - -	Good.	—	Good.

Characters of growth in Sub-culture.

In all cases the same, viz. :—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Copious, easily obtained, rapid growth. Wrinkled.	Easily obtained, rapid extension. Film ; thick, white, opaque, and generally thrown into folds.

H 23. J.P.

Lung from a man aged 41 years.

Source of Cultures.	Sown.
The prepectoral gland of Calf 365 - - - - - -	19.v.04.
The prescapular gland of Calf 421 - - - - -	8.viii.04.
The prepectoral gland of Calf 411 - - - - -	9.viii.04.
The prepectoral gland of Calf 431 - - - - -	19.ix.04.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.	Dextrose Serum.
Calf 365 - - -	—	Good, 25 days.	Good, 25 days.
Calf 421 - - -	Poor, decayed.	—	—
Calf 411 - - -	Very good.	—	—
Calf 431 - - -	Good in 29 days.	—	—

Characters of growth in Sub-culture.
In all cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Very good, easy and rapid.	Film is thick and cream coloured. Growth easily obtained.

H 25. A.T.

Lung of a female aged 30 years.

Source of Cultures.	Sown.
G.P. 1282 from original material	1.vi.04.
Prescapular gland of Calf 417	5.viii.04.

Characters of growth in Primary Culture.

G.P. 1282.	On serum	- -	Copious.
Calf 417.	„ „	- -	Moderate growth.

Characters of growth in Sub-culture.
In both cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very copious.	Very copious.	Copious.	Very easily obtained, rapid extension, thick and forced.

One of the most easily, luxuriantly and rapidly growing bacilli.

H 26. K.M.

Kidney removed by operation from a female 25 years old.

Source of Cultures.	Sown.
G.P. 1326 from original material - - - - - - - -	17.viii.04
The prepectoral gland of Calf 437 - - - - - - -	29.ix.04

H 26. K.M.—*contd.*

Characters of growth in Primary Culture.

On Serum - - Good in 22 and 34 days respectively.

Characters of growth in Sub-culture.

In both cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Good ; delay very small.	Easily obtained ; thick, white and nodular film.

H 27. B.D.

Cervical Glands of a child aged 11½ years removed by operation.

Source of the Culture.	Sown.
G.P. 1216 inoculated with human material - - - - -	2.vii.04

Characters of growth in Primary Culture.

On serum - - - Luxuriant in 27 days.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Luxuriant.	Very good ; thick, white and wrinkled, and very easily grown.

Note.—One of the most easily and luxuriantly growing bacilli examined.

H 28. C.L.

Cervical Glands of a child aged 1$\frac{2}{12}$ years removed by operation.

Source of Cultures.	Sown.
The human cervical glands - - - - - - -	12.v.04.
Rabbit 102 from G.P's. 271–272 inoculated with the human material - - - - - - -	20.vii.04.
G.P. 341 from Calf 513 (prescapular gland) - - -	6.viii.04.
Prescapular gland of Calf 515 - - - - -	12.viii.04.
Rabbit 123 from Calf 515 - - - - - -	22.ix.04.
G.P. 400 from the milk of Cow 143 - - - -	18.x.04.
Kidney of Goat 23 - - - - - - - -	16.xi.04.
Cervical glands of Monkey 63 - - - - -	12.xii.04.

Characters of growth in Primary Culture.

In all cases the same, viz. :—

On serum the growth ultimately obtained was relatively scanty, and as a rule somewhat slow in appearing.
On dextrose serum scanty and colourless, and slow growing.
On glycerin serum no growth was obtained.

H 28. C.L.—*contd.*

Characters of growth in Sub-culture.

In all cases the same, viz. : —

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Scanty and slow growing.	Scanty and slow growing. Appearance of growth much delayed in early sub-cultures.	Very scanty and very slow growing. Appearance of growth delayed for some weeks and often not obtained in early sub-cultures.	Scanty growth. Generally difficult to obtain in early sub-cultures.

The cultures from the goat and rabbits were somewhat more eugonic than those from other sources.

H 29. M.F.

Cervical Glands from a child aged 7½ years removed by operation.

Source of the Cultures.	Sown.
G.P. 1329 from the human material - - - - - - -	30.vii.04.
G.P. 1334 „ „ „ „ - - - - - -	8.viii.04.
Thoracic gland of Calf 477 - - - - - - -	2.ix.04.
Rabbit 115 from G.Ps. 1328–1329 - - - - - -	22.ix.04.
G.P. 1462 from liver of Calf 663 born of the infected heifer 251 -	28.i.05.

Characters of growth in Primary Culture.

G.P. 1329.	Colourless, scanty growth on dextrose serum, visible in 9 days.
— 1334.	On serum, growth was visible in 9 days and ultimately became fairly copious.
Calf 447.	On serum, a scanty growth in 2 months.
Rabbit 115.	On serum, a good growth in 6 weeks.
G.P. 1462.	Original cultures raised on serum.

Characters of growth in Sub-culture.

In all cases the same, viz. :—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good : fairly rapid.	As on serum.	Slow, delayed and poor.	Moderate yield, and obtained only with some difficulty.

H 30. E.M.

Mesenteric and Bronchial Glands from a child $3\frac{10}{12}$ years.

Source of Cultures.	Sown.
G.P. 1338 from G.P.'s 284–285 (original material) - - - -	5.viii.04.
G.P. 1337 „ „ „ - - - -	8.viii.04.

Characters of growth in Primary Cultures.
Good in 12 days on serum.

Characters of growth in Sub-culture.
In both cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Luxuriant.	Very good, rapidly growing, thick, white and wrinkled.

One of the most luxuriantly and rapidly-growing bacilli examined.

H 31. L.F.

Cervical Glands of a child, aged 7 years, removed by operation.

Source of Culture.	Sown.
*Human cervical glands.	24.ix.04.

Character of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good : fairly rapid.	As on serum.	Poor : growth delayed for some weeks.	Moderately thin, slow growing and capricious.

H 32. Y.W.

Lung, Bronchial Gland and Mesenteric Glands from a child aged $2\frac{9}{12}$ *years.*

Source of Cultures.	Sown.
1. Mesenteric gland :	
† The human mesenteric glands	1.x.04.
Prescapular gland of Goat 7	19.xi.04.
Thoracic gland of Calf 465	9.xi.04.
2. Bronchial gland :	
Rabbit 166 from the human material	6.i.05.

Characters of growth in Primary Culture.

Source.	Serum.	Serum Agar.	Glycerin Serum.
Original material	Scanty and colourless	—	—
Goat 7	Moderate growth	Very scanty and colourless	—
Calf 465	Very scanty and colourless	Nil 64 days	Scanty: nearly colourless, discrete colonies.
Rabbit 166	Moderate growth	—	—

Characters of growth in Sub-culture.

In all cases the same, viz. :—

Source.	Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
All "Wilson" Cultures	Good and fairly rapid.	Good and fairly rapid.	In early cultures no growth for nearly three months ; ultimate yield poor.	Growth delayed, film thin.

Cultures from the goat and rabbit were slightly more eugonic than from the other sources.

H 33. R.T.

Axillary Gland from a child aged 7 years.

Source of Culture.	Sown.
The human axillary gland	17.viii.04.

H 33. R.T.—*contd.*

Characters of growth in Primary Culture.
Very good on serum and on serum agar.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Copious, thick and white.	Easily obtained and rapidly growing. Film thick.

H 34. C.U.

Cervical Glands. Details unknown.

Source of Culture.	Sown.
* G.P. 236 from original material - - - - -	4.i.03.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Very good.	Easily obtained, thick, and folded.

H 35. C.B.

Bronchial Gland of a child aged 5½ years.

Source of the Culture.	Sown.
* G.P. 571 inoculated with the human material - - - -	23.vi.03

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Fairly easily growing and moderate yield.	Fairly easily obtained, moderately thick opaque film.

H 36. M.D.

Lung and Mesenteric Glands from a child 4½ years old.

Source of the Culture.	Sown.
The human mesenteric glands - - - - - - -	11.i.05
G.P. 1571 inoculated with the human lung - - - -	3.iii.05.

Characters of growth in Primary Culture.

Source.	Serum.	Glycerin Serum.
Mesenteric gland direct from the human material - -	Very scanty, 31 days	—
Lung through G.P. 1571 - - - - - - -	Failed	Very copious, 21 days.

H 36. M.D.—*contd.*

Characters of growth in Sub-culture.

Cultures from the lung and from the mesenteric glands had similar characters, viz.:—

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant	Luxuriant	Very good	Very good, thick, white, folded pellicle.

H 38. J.M.

Cervical Glands from a child aged 1 *year and* 3 *months.*

Source of Culture.	Sown.
G.P. 793 from original material - - -	9.xi.03.

Characters of growth in Primary Culture.

On Serum - - Good.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good.	Good.	Fair. Much more copious after being in culture for 12 months.	Film thin but obtained fairly easily after being in culture many months.

H 39, M.B.

Cervical Glands of a child, aged 12 *years, removed by operation.*

Source of culture.	Date of sowing.
* The human material	22.x.04.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Good.	Good.	Good.	Easily obtained ; fairly thick film.

H 40. J.G.

A Testis with Epididymis of a man aged 43 *years.*

Source of the Culture.	Sown.
*G.P. 1439 from human material - - - - - - -	9.xii.04

Characters of growth in Primary Culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good	Very good	Good, but rather slow	Fairly thick, and obtained with little difficulty.

H 41. A.S.

Synovial Membrane from a knee-joint of a female aged 39 years.

Source of Culture.	Sown.
†G.P. 1435 from original material - - - - - - -	7.i.05
†G.P. 1437 „ „ „ - - - - - - -	18.xii.04

Characters of growth in Primary Culture.

G.P. 1435 on serum - - - Good
G.P. 1437 „ „ - - - Good

Characters of growth in Sub-culture.

In both cases the same :

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Very good, easy and rapid.	Easily obtained, rapid extension and film of moderate thickness.

H 42. M.R.

Scrapings from a Tuberculous ankle-joint from a female aged 64 years.

Source of Culture.	Sown.
† G.P. 447 from original material.	24.xii.04.

Characters of growth in Primary Culture.

On Serum - - - Good.
On Glycerin Serum - Good.

Characters of growth in Sub-Culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Copious, easy and rapid.	Growth easily obtained, rapid extension and thick film.

One of the most eugonic cultures in the laboratory.

H 43. F.F.

Synovial Membrane of a knee-joint from a child aged 7 years.

Source of the Culture.	Sown.
†G.P. 1425 inoculated with human material - - -	12.i.05.

Characters of growth in Primary Culture.

A poor growth on serum in 3 weeks.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Good.	Easily obtained, film of moderate thickness.

H 44. D.C.

Cervical Glands of a child aged 4 years removed by operation.

Source of the Culture.	Sown.
* The human material - - - - - - -	8.xi.04

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Very good. Well marked growth in 7 days.	Very good, film thick, fairly easily and rapidly growing.

H 45. F.M.

Bronchial Gland from a child aged 1 year.

Source of Culture.	Sown.
G.P. 1137 from original material - - - - - - -	10.v 04

Characters of growth in Primary Culture.

On Serum—Good in 12 days.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Luxuriant.	Luxuriant.

One of the most easily, rapidly, and luxuriantly-growing bacilli examined.

H 46. H.W.

Caseous Material from the Patella of a Tuberculous knee-joint.

Source of Culture.	Sown.
G.P. 1444 from human material.	12.i.05.

Character of growth in Primary Culture.

G.P. 1444	On serum, poor. On Glycerin serum, nil.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Copious.	Copious.	Growth occurred without any delay, was rapid and very good.	As in the case of agar, film thick.

H 47. S.B.

Pus and fragments of Bone from the Hip-joint of a child aged 6½ years.

Source of Culture.	Sown.
† G.P. 1430 inoculated with the human material - - - -	6.i.05.

Characters of growth in Primary Culture.

On Serum, thick opaque masses of growth in 14 days.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Luxuriant.	Luxuriant.	Very good. Well marked growth in 7 days.	Thick and easily grown: sometimes thrown into folds.

H 48. W.P.

Lung of a man aged 40 years.

Source of Culture.	Sown.
G.P. 1494 from original material - - - - -	10.iv.05.
Rabbit 301 „ „ „ - - - - -	10.iv.05.

Characters of growth in Primary Cultures.

G.P. 1494. On serum, good in 9 days.
Rabbit 301. „ „ copious growth in 22 days.

Characters of growth in Sub-culture.

In both cases the same.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very luxuriant.	Very luxuriant.	Very good, thick, wrinkled, white, with small nodules.	—

H 49. T.C.

Mesenteric Glands of a male aged 18 years.

Source of the Culture.	Sown.
The human mesenteric glands - - - - - - .	6.iv.05.

Characters of growth in Primary Culture.

Sown on serum, but contaminated so that the characters of the primary growth were obscured. Microscopic evidence of growth at the end of four days.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Grows after some delay, and yields only a moderate growth.	Moderately thick film, fairly easily started, but capricious.

H 50. P.H.

Lung of a male aged 34 *years.*

Source of Culture.	Sown.
* G.P. 1490 from human material.	27.iii.05.

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very luxuriant.	Very luxuriant.	Very good ; thick white, nodular and wrinkled.	—

H 53. D.H.

Scrapings of tissue removed by operation from a case of Lupus Vulgaris, occurring in a girl aged 14 *years.*

Source of Culture.	Sown.
* G.P. 1482 from human material.	8.iii.05

Characters of growth in Sub-culture.

Serum.	Glycerin Serum.	Glycerin Agar.	Glycerin Broth.
Very good.	Very good.	Good and obtained after but little delay.	—

July 18*th*, 1905.

H. J. HUTCHENS.

REPORT ON THE CHANGES IN REACTION PRODUCED IN BROTH BY HUMAN AND BOVINE TUBERCLE BACILLI.

Dr. A. STANLEY GRIFFITH.

ON THE CHANGES IN REACTION PRODUCED IN GLYCERIN BROTH BY HUMAN AND BOVINE TUBERCLE BACILLI.

INTRODUCTION.

In a paper published in 1903[*] Theobald Smith described a simple method of distinguishing the bovine from the human type of bacilli in broth cultures.

He found that if bovine bacilli are grown on the surface of 3 to 5 per cent. glycerin broth, the acidity of which to begin with is about 2 per cent. the broth becomes steadily less and less acid until finally, when the membrane is complete, an acidity or an alkalinity of about 0·1 to 0·2 per cent. is reached; with human bacilli on the other hand the acidity during the formation of the membrane diminishes at first, falling sometimes as low as 0·3 per cent., then, as the membrane enlarges and thickens, gradually rises again and may reach 2 per cent., but more commonly fluctuates between 0·5 and 1·5 per cent.

Smith claimed that the differences thus brought out established a difference in physiological properties.

This conclusion was based upon the results of the investigation of six strains of tubercle bacilli of human origin and six strains of tubercle bacilli of bovine type; four of the latter were obtained from the bovine, one from a horse and one from a cat; one of the strains of human origin was virulent for calves and rabbits.

Subsequently[†] three more cultures isolated from the human being were tested after this method; these cultures were found to pursue one of two courses; they either attained a greater than the initial acidity at the end of two to three months, after having first become less acid, or else (as occurred with Human XIII.) the reaction remained after some preliminary fluctuation at an intermediate level of acidity.

Bacilli having the character of human bacilli were found therefore to differ among themselves in the degree of acid production; this phenomenon was constant for the same culture.

In a more recent publication[‡] the results of experiments with varying amounts of glycerin are given. With bovine tubercle bacilli the percentage of glycerin had no influence on the final reaction. With slightly virulent human bacilli (except Human XIII.) glycerin up to $\frac{3}{10}$ per cent. did not interfere with a final slightly alkaline reaction, but in broth containing 1 to 2 per cent. the reaction remained slightly acid; in broth containing 3 to 5 per cent. a considerable amount of acid accumulated after several months.

With the human culture mentioned above which produced only a moderate amount of acid, all the broths containing up to 1 per cent. became alkaline; the flask containing 2 per cent. approached and nearly attained the neutral level, but the 3 per cent. broth remained acid.

From these experiments Theobold Smith concluded that in order to bring out the differences at least 3 per cent. glycerin should be employed.

As the method, described in Theobald Smith's first communication on this subject, appeared to supply a valuable means of distinguishing bovine from human tubercle bacilli whenever isolated in pure culture, I was directed by the Commission to repeat Smith's experiments with the different strains of tubercle bacilli which we had isolated from the ox and the human being in order to ascertain whether the same differentiation into two types could be made.

The work in connection with the investigation has been carried out at the Royalcot Laboratory.

TECHNIQUE.

The cultures have all been grown in half litre flasks, each containing 200 c.c. of broth.

The broth was prepared from beef in the usual way, and in most of the earlier tests was previously fermented for two days with bacillus coli before adding the peptone, salt and glycerin. The initial acidity of the broth has varied from 10 to 21. Glycerin in the majority of cases was added in the proportion of 2 per cent.; in a few cases flasks containing 2 per cent. and 5 per cent. glycerin have been inoculated at the same time. In all cases chemically pure glycerin has been used.

The samples of broth to be tested were withdrawn from the flasks either by means of a syphon fitted into the neck, or by means of a side tube issuing from the bottom of the flask; the flasks with syphons were put in the incubator in an inclined position on a wooden stand. The glass tube forming the syphon passed to the bottom of the broth through a solid rubber cork in which there was also a short length of glass tubing, plugged with wool, which acted as an air inlet.

Over the external extremity of the syphon tube, as well as over that of the side tube, a short length of semi-pressure tubing was placed. In the distal end of the rubber tube a short glass cannula was inserted; the latter passed through a rubber cork fitted into a small test tube; the rubber tubing was clamped with a spring clamp.

Before withdrawing the test sample sufficient broth was withdrawn to empty the syphon or side tube. After removing the sample the small glass cannula was dipped in 2 per cent. lysol to prevent organisms which might have settled thereon, when exposed to the air, from growing in the broth left behind in the tube external to the clamp.

The flasks with side tubes possess several advantages over those with syphons; they occupy less space in the incubator and are easier to inoculate; there is, besides, no disturbance of the pellicle as is sometimes the case with the syphon, which necessarily passes through the centre of the pellicle.

The method of titration was similar to that usually employed for determining the acidity of broth; 2 c.c. of the broth were diluted to 50 or 60 c.c. with distilled water, and after the addition of 0·5 c.c. of a 0·5 per cent. solution of phenol-phthalein in 50 per cent. alcohol, brought to ebullition; an $\frac{N}{50}$ solution of NaOH was then run in until the liquid had assumed a faint but definite pink colour throughout. In every instance two, and often three or more, separate estimations were made and the average taken.

An attempt was at first made to estimate the amount of growth by a gravimetric method; the broth was filtered through a weighed filter paper and the growth collected: the filter was dried in the incubator for three days and then weighed. This method was found to give unreliable results and was abandoned for the volumetric method; this was carried out in the following way; the growth was collected in a graduated test tube and, after finely subdividing the bacillary masses (with a glass rod), centrifugalised for a definite period; then the level of the column of bacilli was immediately read off.

MATERIAL USED.

The material for inoculating the flasks has in most cases been taken from broth cultures raised directly from serum; in a few cases the bacillus has grown for some generations on broth before transference to the flasks, in others the pellicle which forms on the surface of the condensation water in a serum tube has been used.

I wish here to acknowledge my great indebtedness to Dr. Eastwood for allowing me the use of his broth cultures, and for having had in many cases broth cultures specially raised for me; in this way the labour of the investigation has been considerably lessened, and I have had the advantage of always using young vigorous cultures.

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[*] Trans. Assoc. Amer. Phys., Vol. XVIII., 1903, and The Journ. of Med. Research, Vol. XIII., No. 3, 1905.

[†] Amer. Journ. of the Medical Sciences, Vol. CXXVIII., 1904.

[‡] The Journ. of Med. Research, Vol. XIII., No. 4, 1905.

DISCUSSION AND INTERPRETATION OF EXPERIMENTS.

The Characteristic Reactions of Bovine and Human Cultures.

The results of the experiments are exhibited in tabular form. Considering first the results obtained with the bovine tubercle bacillus, an inspection of the table shows that all the strains have caused a diminution in the acidity of the broth. In four instances the broth became neutral or nearly neutral to phenol-phthalein. In the others the final reaction varied from + 5 to + 11·5. The total reduction in acidity, *i.e.*, the difference between the initial and the final reaction has varied from 1·5 to 12·5, None of the broths which began with an initial acidity of about + 20 became neutral to phenol-phthalein, the final reaction in these cases never attaining a lower level than + 8·5. In several cases the final reaction of the broth was higher than the lowest recorded reaction; this return to a higher acidity was in no instance great and did not exceed + 2·5.

The virulent strains of human origin have with one exception also caused a diminution in the acidity of the broth. The greatest diminution occurred in the case of H. 28 "C.L." which yielded the least amount of growth. None of the cultures brought the reaction of the broth to the phenol-phthalein neutral point, the final reaction varying between + 1·5 and + 13·7; in some of the strains the diminution in the acidity was very slight. As in the case of bovine strains the final reaction was on several occasions slightly higher than the lowest recorded reaction.

The exception mentioned above occurred in the case of H.19 "S.W." This strain grew slowly but finally yielded an abundant growth; the final acidity of the broth was + 4·5 degrees higher than the initial.

All the slightly virulent strains first caused a diminution in the acidity of the broth (this diminution was not observed in every case on account of the first tests not being made soon enough) and then an increase, the final acidity in the majority of the cases being higher than the initial. This excess of the final over the initial acidity varied from + 0·5 to + 17. In four instances the final acidity was slightly less than, and in one the same as the initial acidity.

Reactions with Viruses Exhibiting Experimental Variations.

In several instances both with human and bovine viruses strains of tubercle bacilli have been isolated from experimental animals which have differed in cultural characters and pathogenic effects from the first cultures of the virus isolated; and in one case where the virus consisted of mixed sputum two different kinds of bacilli have been isolated from animals infected with the original material.

The results with these strains I shall consider separately; they show that the differences in cultural luxuriance are accompanied by a corresponding difference in reaction.

In the case of H.16 J.H. the slightly virulent culture isolated from Calf 273 on two occasions caused the final acidity of the broth to be higher than the initial. The two virulent strains of this virus which have been tested both reduced the acidity of the broth; the strain from Calf 559 grew better than that from Bull 423A., and it is interesting to note that the diminution in acidity was not so great.

In the case of H.17 Sp. B., the slightly virulent culture isolated from Calf 265 caused once an increase and once a slight diminution in the final acidity of the broth; another slightly virulent strain (Calf 339) caused a slight increase in the acidity of the broth. Three virulent strains of this virus have been tested; the cultures from Calf 571 and Calf 555 steadily diminished the acidity of the medium; the latter grown on an unfermented broth (reaction + 17·5) caused a greater diminution in acidity than the former grown on a fermented broth (reaction + 10); in this case (Calf 555) the diluted broth in the cold became quite pink on the addition of phenol-phthalein. The strain from Rabbit 181 was tested four times, twice on fermented broth and twice on unfermented; on the fermented broth the final reaction was on both occasions slightly less than the initial; on the unfermented the final acidity was higher than the intial; it may be mentioned that the culture used for the fermented broth was of an earlier generation than those used for the unfermented and moreover did not grow quite so abundantly.

Four strains of H.2. Sp. A. have been tested; one, a slightly virulent culture isolated from Calf 111, rendered the broth on two occasions more acid; the other three, all virulent cultures, caused a slight reduction in acidity.

In the case of H. 13 "A. D." only one culture, that from Calf 301, has been investigated; this produced an acid reaction in the broth; two attempts have been made to grow the culture obtained from Rat 15, but without success; attention may here be drawn to the luxuriant growth obtained with this strain on glucose broth

In two instances the bovine tubercle bacillus has apparently been transformed by passage through the body of a dog into a bacillus having the cultural characters of a Group II. organism

The culture isolated from Dog 18, Virus B. IV. produced a moderately thick pellicle, but unfortunately before the test was completed the broth became contaminated.

The culture isolated from Dog 50, Virus B. IX. produced an acid reaction in the broth.

Reactions obtained under Conditions not strictly Comparable.

The results of certain tests are not included in the tables because of the exceptional conditions under which they were obtained.

These experiments will be described in detail.

Virus B.I. (Guinea-pig 14) was sown on a flask containing a syphon; at the end of 133 days very little growth had taken place, so the syphon, being required for another flask, was removed and replaced by a solid rubber cork. After five weeks further incubation, active multiplication suddenly set in, and in a very short time the whole surface of the broth was covered. The final reaction of the broth was 4 per litre more acid than the initial reaction [*See* page 63.]

Four strains of human origin, three slightly virulent and one virulent for calves and rabbits, were grown on a fermented broth which took several days to filter; when at last filtered clear, the broth was considerably diminished in bulk, and the loss was made up with distilled water before adding the peptone, salt and glycerin.

The broth was evidently unfavourable for the full development of the cultures, and the pellicle formed in each case was thin, grey and translucent, and of about the thickness of ordinary tissue paper; the acidity of the broth was also in each case steadily reduced and in about equal degrees.

The results are important as showing that when slightly virulent tubercle bacilli produce thin membranes, they do not react differently from virulent strains.

Interpretation of Results.

Reviewing the experiments as a whole it is found that all the strains of tubercle bacilli used in the investigation whether of human or bovine origin, first casused a diminution in the acidity of the broth.

In a few cases this diminution steadily progressed until the broth became neutral, or nearly neutral, to phenol-phthalein.

In other cases after a preliminary diminution in acidity, varying in amount, but never great enough to cause the medium to become neutral to phenol-phthalein, the original acidity of the medium was restored and in many cases surpassed.

Besides these cultures, there are others which have produced all intermediate varieties of reaction; some have steadily reduced the acidity of the broth to a varying extent, but not to the phenol-phthalein neutral point, others after first diminishing the acidity have shown all degrees of return from the lowest point reached to the initial acidity of the broth. If these results are arranged in numerical order, beginning with the strain which has reduced the acidity to the greatest extent and ending with the strain which has produced the greatest amount of acid, it will be found they form a continuous series in which there is no gap indicating that two wholly distinct kinds of bacilli are being dealt with.

Examined with reference to the virulence of the organisms such a series will show that, although there is no sharp line of demarcation, if a line be drawn at a point where the initial and final reaction are the same, all the strains above the line with few exceptions possess high virulence, whilst all the strains below the line with one exception have only slight virulence for the calf and rabbit.

As will be seen from the descriptions of the characters of the pellicles the great majority of the strains which caused a marked diminution in the acidity of the medium produced much less bulky membranes than those which increased the acidity of the medium.

Virulent tubercle bacilli, as is well-known, grow with more difficulty and ordinarily produce on broth much thinner pellicles than the slightly virulent human bacillus.

The slightly virulent strains on the other hand grow sometimes rapidly, sometimes slowly, but always on a favourable medium finally produce an opaque, wrinkled, or warty membrane often of considerable density.

In the latter cases the amount of growth was obviously greater than that produced by the majority of the bovine bacilli. It was for this reason that an estimate of the amount of growth was made in order to ascertain whether there was any correspondence between it and the change in reaction.

These estimations (volumetric only) together with the corresponding change in reaction are given in tabular form. The table shows that, although the change in reaction is not strictly in accordance with the amount of growth, generally when the growth has been scanty the medium has become less acid, and when the growth has been abundant more acid.

It has been observed in these experiments, and it has also been pointed out by Theobald Smith, that in the case of the slightly virulent bacillus acid accumulates very rapidly towards the end of the experiment. In several instances it has happened that after more than two months' growth the acidity of the medium has been only about equal to, or even less than, the initial acidity, but when tested after the lapse of a few more weeks there was found to have been a sudden rise in the acidity, although no further increase in the thickness of the pellicle could be definitely said to have taken place.

The rise of acidity does not appear to be throughout the experiment in exact proportion to the amount of growth.

It is clear, therefore, that in estimating the relationship between cultural luxuriance and change in reaction another factor, *i.e.*, duration of growth, besides total amount of growth, must be taken into consideration. The growth of the bovine tubercle bacillus stops much sooner than that of the human bacillus; yet both at first react in the same way and if, as has occurred in three cases, the human bacillus ceases to grow when the pellicle formed is still thin and translucent, the change in reaction is not different from that produced by a virulent bacillus growing at the same rate.

Moreover, when virulent strains grow well and produce moderately thick pellicles the diminution in acidity is not so great as when thin membranes are formed. Were it true that all virulent strains caused an alkaline change only in the broth, those virulent strains which have produced moderately good growths ought to have reduced the acidity of the broth to a far greater extent than those which grew scantily. My results clearly show that this greater effect has not followed, and none of the bacilli of Group I. possessed of high virulence, which have grown well have made the broth neutral to phenolphthalein.

In fact, in many instances, the diminution in acidity has been only a very slight one and in one instance when the growth was specially abundant the final reaction was 4 per litre more acid than the initial.

It seems to me that these experiments at any rate prove conclusively that there are strains of tubercle bacilli possessed of high virulence, which have the power, equally with the slightly virulent bacillus, of producing acid out of glycerin. As regards the virulent strains which have reduced the acidity of the medium to a marked degree it would be impossible to say, from information obtained solely by the method of titration, whether acid was or was not produced during the growth of the bacillus; this could only be determined by exact chemical analysis. My own opinion is that in these cases also, except perhaps when the growth was extremely thin, a certain amount of acid, varying with the amount of growth, was formed, which was masked by a much greater production of alkali. This view is based upon the fact brought out by my experiments that the diminution in acidity is less pronounced when the growth is moderate in amount than when it is very scanty.

SUMMARY AND CONCLUSION.

The investigation has shown that when virulent tubercle bacilli, whether of human or bovine origin, are grown on the surface of glycerin broth the acidity of the medium is gradually diminished, the final acidity being less than the initial; the diminution is variable in amount and has in general been greatest when the growth was scantiest.

On the other hand when slightly virulent tubercle bacilli are grown on the surface of glycerin broth the acidity of the medium is at first diminished; then as the pellicle increases in thickness the acidity gradually rises again, the final acidity being higher than the initial. To this general rule exceptions have occurred—for example, the final acidity with bacilli of Group I. has on one occasion exceeded the initial, whilst that in the case of bacilli of Group II. has sometimes been less than the initial—and the distinction which otherwise might have been drawn between the virulent and slightly virulent viruses, the former reducing, the latter increasing the acidity of the medium, is thereby abolished. Yet if no exceptions had occurred it would be incorrect to assert that the distinction so drawn signified a difference in physiological properties; since we cannot neglect variations in reaction attributable merely to differences in amount of growth.

Although the change in reaction has not in every case been in strict accordance with the amount of growth, some correspondence between the two can be noted.

When an organism grows luxuriantly the final acidity of the broth is usually higher than the initial; when an organism grows scantily the reduction in acidity is more pronounced than when the growth is moderate in amount.

My observations therefore clearly show that glycerin broth does not differentiate tubercle bacilli into two classes, and yield no evidence that there is an essential difference in the action of different strains of tubercle bacilli on glycerin.

COMPARISON WITH THEOBALD SMITH'S RESULTS.

I am able to confirm Theobald Smith in his general statements that the slightly virulent tubercle bacillus of human origin, when grown upon the surface of glycerin broth, first causes a diminution in the acidity of the medium and then an increase, and that there are strains of tubercle bacilli of bovine origin which steadily diminish the acidity of the broth.

My results do not, however, support the conclusion he draws from his experiments—that the differences he has observed point to a difference in physiological properties.

This conclusion is based upon the investigation of a relatively small number of strains, six human and six bovine; yet if the results as set out in his table be analysed and arranged numerically in a series according to the differences in each case between the initial and final reaction, they form, as Dr. Eastwood has pointed out, an unbroken series in which there is nowhere a gap that would justify the conclusion that two essentially different kinds of organisms were dealt with.

In Theobald Smith's paper no mention is made of the amount or character of the final growth, so that it is not possible to institute exact comparisons between his experiments and mine; the only data given are in the case of a virulent organism, Human XI., which always grew scantily and on three occasions failed completely to cover the surface. It is the experience of most observers that virulent tubercle bacilli grow less readily and generally produce a smaller volume of growth than slightly virulent bacilli.

When two related organisms, such as the colon bacillus and the hog cholera bacillus, growing equally rapidly and vigorously, produce in a liquid medium containing a certain sugar, one an acid reaction, the other an alkaline one, differences in biochemical action may be said to have been demonstrated, but it would be incorrect to assert on similar grounds that physiological differences exist between two strains of tubercle bacilli when one produces a feeble membrane which covers only a part of the surface, while the other produces a membrane of considerable density.

In his most recent paper Theobald Smith recommends that in order to bring out the difference between the two kinds of organisms at least 3 per cent. glycerin should be employed; he found that, whereas the percentage of glycerin (traces up to 5 per cent.) had no influence on the final reaction in the case of the bovine bacillus, in the case of the human bacillus acid was only produced in marked degree when 3 to 5 per cent. glycerin was present.

In my experiments with bacilli of low virulence the final acidity has rarely been lower than the initial and this in spite of the fact that the broth I have used has contained 2 per cent. glycerin only. It is indeed noteworthy that the amount of acid produced by the slightly virulent organisms on 2 per cent. glycerin has in general been very much greater than in Theobald Smith's experiments in which 3 to 5 per cent. glycerin was used.

TABLE I.

SUMMARY OF RESULTS WITH TUBERCLE BACILLI OF BOVINE ORIGIN.

Virus and Strain.		Initial reaction of broth.	Lowest recorded reaction.	Final reaction.	Difference between final and initial reaction.	Characters of pellicle.
B I.	**Cow 44** -	+12·5	0·0	Neutral or faintly alkaline.	- 12·5	Thin and translucent.
,,	,, -	+20	+10·	+11·	- 9·	Thin greyish white and lacelike.
B II.	**Calf 134** -	+21·	+10·6	+10·6	- 10·4	Partly opaque and white, partly thin and filmy.
,,	,, -	+21·	+ 8·	+ 8·5	- 12·5	Thin grey and almost transparent, with a small warty island.
B IV.	**Direct** - -	+11·	+2· or 1·	+2· or 1·	-·9 or 10·	Thin grey and translucent.
,,	,, -	+17·	+11·	+11·	- 6·	Grey translucent membrane of the thickness of ordinary tissue paper.
B V.	**Goat 2** - -	+21·	+10·	+10·3	- 9·7	Moderately thick opaque white pellicle.
,,	,, - -	+13·	+ 5·	+ 5·	- 8·	Opaque white pellicle of moderate thickness.
,,	,, - -	+12·	+ 6·25	+ 7·	- 5·	Opaque whitish islands coverin not more than half the surface.
B V.	**Heifer 80** -	+11·	+ 7·	+ 8·5	- 2·5	Thin grey and translucent.
B VIII.	**Direct** - -	+17·	—	+13·5	- 6·	Thin grey membrane with opaque whitish patches covering about half the surface.
B IX.	**G.P. 1069** -	+21·	+10·2	+11·	- 10·	Greyish translucent speckled membrane which sank at the end of the fifth week.
B IX.	**Lemur 2** -	+17·5	+11·5	+11·5	- 5·5	Partly opaque white and slightly warty, partly thin and translucent; the whole of the pellicle had sunk at the end of the 38th day.
B IX.	**Dog 60** -	+17·5	+15·	+16·	- 1·5	Thin membrane which sank at the end of the 4th week.
B X.	**G.P. 1070** -	+20·	+13·5	+11·5	- 8·5	Thin and grey and studded with opaque raised colonies.
B XI.	**Rabbit 66** -	+21·	+ 8·	+10·5	- 10·5	Partly greyish-white semi-opaque, and studded with raised colonies, partly thin and translucent.
B XIII.	**Rabbit 116** -	+10·	+ 1·	+ 3·	- 7·	Very thin.
B XIV.	**Direct** - -	+17·	+11·	+11·	- 6·	Thin grey membrane with opaque warty patches, covering a little more than half the surface.
B XV.	**Direct** - -	+10·	+ 4·5	+ 7·	- 3·	Resembled ordinary tissue paper.
B XVIII.	**Direct** -	+10·	+ 1·	+ 3·	- 7·	Very thin.

TABLE II.

SUMMARY OF RESULTS WITH TUBERCLE BACILLI OF HUMAN ORIGIN.

(*a*) Virulent Strains.

Virus and Strain.	Initial reaction of broth.	Lowest recorded reaction.	Final reaction.	Difference between initial and final reaction.	Characters of pellicle.
H 7. "C.M." Cow 73	+21·	+ 8·3	+ 9·6	-11·4	Thin translucent membrane containing a few opaque patches.
H 7. "C.M." Cow 73	+20·	+ 9·	+13·7	-63·	Thin grey and translucent with thicker white patches.
H 10. "B.S." Heifer 231	+13·	+ 3·75	+ 3·75	- 9·25	Thin grey and translucent with some opaque white patches.
H 14. "F.S." Calf 125	+13·	+ 3·5	+ 5·	- 8·	Opaque warty islands surrounded by a delicate grey membrane.
H 16. "J.H." ,, 559	+13·	+ 4·5	+ 5·25	- 7·75	Broken up into islands, some thin and grey, others thicker and more opaque.
H 16. "J.H." ,, 423a	+13·	+ 3·75	+ 3·75	- 9·25	Thin and filmy, with three opaque islands.
H 19. "S.W." Heifer 239	+17·5	—	+22·	+ 4·5	Resembled heavy lace.
H 28. "C.L." Direct -	+14·	+ 1·5	+ 1·5	-12·5	Almost transparent except for two small opaque patches.
H 29. "M.F." G.P. 1334	+17·5	+14·	+14·	- 3·5	Partly thick yellow and warty; partly greyish-white and translucent.
H 31. "L.F." Direct -	+14·	+ 6·5	+ 6·5	- 7·75	Surface half covered with moderately thick white pellicle; rest with a few delicate grey islands.
H 32. "Y.W." Direct Mesenteric glands.]	+13·	+ 4·5	+ 7·	- 6·	Thin and grey with a few opaque whitish patches.
H 38. "J.M." G.P. 793	+15·	+ 7·	+ 7·	- 8·	Thin grey and translucent.
H 49. "T.C." Direct -	+13·	+ 9·5	+11·	- 2·	Moderately thick.
H 49. "T.C." Direct -	+13·	+11·	+11·	- 2·	Moderately thick.
H 53. "D.H." G.P. 1482	+20·	+ 8·5	+ 8·5	-11·5	Thin grey and translucent, with thicker white patches.

(*b*) Slightly Virulent Strains

Virus and Strain.	Initial reaction of broth.	Lowest recorded reaction.	Final reaction.	Difference between initial and final reaction.	Characters of pellicle.
H 2. “Sp. A.” Calf 111	+ 15·	+ 7·	+ 18·	+ 3·	Thick creamy-white and wrinkled.
H 2. “Sp. A.” Calf 111	+ 20·	+ 16·5	+ 23·5	+ 3·5	Opaque white and wrinkled.
H 8. “S.C.” Direct	+ 11·5	+ 9·6	+ 18·	+ 6·5	Partly thick opaque and white, partly thinner and translucent.
H 9. “C.T.” Calf 183	+ 10·5	+ 6·75	+ 10·5	0·0	Moderately thick, greyish-white and wrinkled.
H 11. “E.D.” Calf 221	+ 11·5	+ 10·5	+ 18·	+ 6·5	Partly thick and opaque white partly thinner and more translucent.
H 12. “H.N.” Calf 123	+ 17·	—	+ 24·5	+ 7·5	Thick creamy-white and wrinkled.
H 13. “A.D.” Calf 301	+ 15·	+ 8·5	+ 20·	+ 5·	Thick creamy-white and wrinkled.
H 15. “I.W.” Calf 311	+ 10·	+ 7·	+ 18·	+ 6·5	Thick white warty growth.
H 16. “J.H.” Calf 273	+ 11·5	+ 5·	+ 18·	+ 6·5	Thick creamy and wrinkled.
H 17. “Sp.B.” Calf 265	+ 10·	+ 7·	+ 14·	+ 4·	Moist whitish and slightly wrinkled.
H 17. “Sp.B.” Calf 265	+ 20·	+ 14·5	+ 17·	- 3·	Greyish-white and wrinkled, finally became broken up into islands.
H 17. “Sp.B.” Calf 339	+ 18·	—	+ 20·5	+ 2·5	Thick opaque white slightly creamy and very warty.
H 18. “T.T.” Calf 131	+ 15·	—	+ 28·	+ 13·	Thick warty and wrinkled.
H 22. “F.W.” Calf 293	+ 17·	+ 17·	+ 23·	+ 6·	Partly yellowish and warty, partly greyish white and wrinkled.
H 23. “J.P.” Calf 365	+ 14·	+ 6·5	+ 13·5	0·5	Partly thick and slightly creamy, partly grey and translucent.
H 26. “K.M.” G. P. 1326	+ 17·5	+ 14·	+ 21·5	+ 4·	Unequal in thickness, greater part being thick creamy and warty.
H 27. “B.D.” G. P. 1216	+ 17·5	—	+ 25·	+ 7·5	For most part thick with warty patches: parts were thin and grey.
H 30. “E.M.” G. P. 1337	+ 13·	+ 8·	+ 18·	+ 5·	Thick creamy and wrinkled.
H 34. “C.U.” G. P. 236	+ 10·	+ 5·5	+ 8·75	- 1·25	Moderately thick opaque white.
H 34. “C.U.” G. P. 236	+ 11·5	+ 9·5	+ 12·	+ 0·5	Thin and grey with a few whitish patches.
H 36. “M.D.” Direct	+ 19·5	+ 13·	+ 27·	+ 7·5	Thick and creamy-white.
H 37. “O.J.” G. P. 1427	+ 17·5	+ 13·	+ 20·5	+ 3·	Unequal in thickness but for the most part thick creamy-yellow wrinkled and warty.
H 39. “M.B.” Direct	+ 17·	+ 17·	+ 22·	+ 5·	Moderately thick greyish-white and very wrinkled.
H 41. “A.S.” G. P. 1435	+ 17·	+ 18·5	+ 22·	+ 5·	Thick creamy-white and wrinkled.
H 42. “M.R.” G. P. 1447	+ 14·	+ 6·5	+ 13·	+ 1·	Moderately thick greyish-white moist and uniform.
H 44. “D.C.” Direct	+ 17·	—	+ 24·	+ 7·	Thick creamy wrinkled and very warty.
H 45. “F.M.” G. P. 1137	+ 11·	+ 7·	+ 18·	+ 7·	Thick creamy and wrinkled.
H 45. “F.M.” G. P. 1137	+ 11·	+ 7·	+ 28·	+ 17	Thick creamy and wrinkled.
H 48. “W.P.” G. P. 1494	+ 10·	+ 6·75	+ 11·	+ 1·	Moderately thick whitish and slightly wrinkled.
H 50. “P.H.” G. P. 1490	+ 10·	+ 6·75	+ 12·	+ 2·	Moderately thick creamy-white and wrinkled.
H 51. “H.M.” Direct	+ 17·	—	+ 22·5	+ 5·5	Moderately thick greyish-white or slightly creamy.
H 52. “T.F.” G. P. 1567	+ 17·	+ 16·	+ 18·	+ 1·	Moderately thick creamy and wrinkled.
H 54. “C.W.” G. P. 1609	+ 13·	+ 12·	+ 15·	+ 2·	Thick wrinkled and warty.
H 55. “R.D.” Direct	+ 13·	+ 10·	+ 11·5	- 1·5	Moderately thick greyish-white and wrinkled.
H 55. “R.D.” Direct	+ 13·	+ 9·5	+ 13·5	+ 0·5	
H 56. “F.T.” G. P. 1682	+ 20·	—	+ 21·	+ 1·	Thick creamy and wrinkled.
H 57. “B.J.”	+ 17·	—	+ 30·	+ 13	Thick creamy and very warty.
H 61. “E.C.” Direct (Mesenteric glands.)	+ 20·	—	+ 21·	+ 1·	Moderately thick opaque white islands not completely covering the surface.

TABLE III.

SUMMARY OF RESULTS WITH VIRUSES, DIFFERENT STRAINS OF WHICH HAVE SHOWN DIFFERENCES IN VIRULENCE.

Virus and Strain.	Initial reaction of broth.	Lowest recorded reaction.	Final reaction.	Difference between initial and final reaction.	Characters of pellicle.
H 16. "J.H." Calf 273* -	+ 11·5	+ 7·	+ 20·	+ 8·5	Thick creamy and wrinkled.
H 16. "J.H." Calf 273* -	+ 11·5	+ 5·	+ 18·	+ 6·5	Thick creamy and wrinkled.
H 16. "J.H." Bull 423a.† -	+ 13·	+ 3·75	+ 3·75	− 9·25	Thin and almost transparent with a few thicker islands.
H 16. "J.H." Calf 559† -	+ 13·	+ 4·25	+ 5·25	− 7·75	Composed of islands, mostly grey and translucent a few opaque and white.
H 17. "Sp. B." Calf 265.* -	+ 10·	+ 7·	+ 14·	+ 4·	Moderately thick moist white and slightly wrinkled.
H 17. "Sp. B." Calf 265.* -	+ 20·	+ 14·5	+ 17·	− 3·	Greyish-white and finely wrinkled; moderately thick.
H 17. "Sp. B." Calf 339.* -	+ 18·	—	+ 20·5	+ 1·5	Thick creamy-white warty and slightly wrinkled.
H 17. "Sp. B." Rabbit 181.‡	+ 13·	+ 9·5	+ 9·5	− 3·5	Opaque milky-white islands, which did not completely cover the surface.
H 17. "Sp. B." Rabbit 181.‡	+ 13·	+ 7·	+ 10·	− 3·	One-third opaque and white, rest thin grey and translucent.
H 17. "Sp. B." Rabbit 181.‡	+ 17·5	+ 17·5	+ 23·5	+ 6·	Moderately thick and wrinkled.
H 17. "Sp. B." Rabbit 181.‡	+ 20·	—	+ 21·	+ 1·	Thick wrinkled and slightly warty.
H 17. "Sp. B." Calf 555.† -	+ 17·5	+ 6·	+ 7·	− 10·5	Thin and grey with some slightly thicker patches.
H 17. "Sp. B." Calf 571.† -	+ 10·	+ 4·75	+ 4·75	− 5·25	Ditto.
H 2. "Sp. A." Calf 111* -	+ 20·	+ 16·5	+ 23·5	+ 3·5	Thick opaque white and wrinkled.
H 2. "Sp. A." Calf 111* -	+ 15·	+ 7·	+ 18·	+ 3·	Thick creamy-white and wrinkled.
H 2. "Sp. A." Heifer 11† -	+ 10·	+ 6·5	+ 9·	− 1·	Very thin.
H 2. "Sp. A." Calf 83.† -	+ 12·	+ 5·5	+ 9·5	− 2·5	Thin grey and translucent.
H 2. "Sp. A." Calf 93.† -	+ 13·	+ 9·	+ 11·5	− 1·5	Thin and grey; the pellicle broke up into islands and in three weeks most of these had sunk; a few remained floating and these gradually became opaque and white.
B IX. Dog 50* - - -	+ 20·	+ 13·5	+ 22·	+ 2·	Thick opaque white and granular.

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* Slightly virulent for bovines.
† Highly virulent for bovines.
‡ Virulent in early generations, slightly virulent in later.

TABLE IV.

Table showing the Relation between the Change in Reaction and the Amount of Growth.

Virus and Strain.		Difference between initial and final reaction.	Volume of growth in cubic centimetres.
H 2. "Sp.A."	**Calf 555**	−10·5	1·3
H 16. "J.H."	**Calf 423a**	− 9·25	1·25
B I.	**Cow 44**	− 9·	2·8
B X.	**G.P. 1070**	− 8·5	2·3
H 38. "J.M."	**G.P. 793**	− 8·	1·7
H 31. "L.F."	**Direct**	− 7·5	1·6
H 26. "K.M."	**G.P. 1326**	− 7·5	1·5
H 7. "C.M."	**Cow 73**	− 6·3	3·3
B IX.	**Lemur 2**	− 6·	1·15
B IV.	**Direct**	− 6·	1·6
B XIV.	**Direct**	− 6·	1·1
H 17. "Sp.B."	**Calf 571**	− 5·25	1·5
H 37. "O.J."	**G.P. 1427**	− 5·	1·6
B VIII.	**Direct**	− 3·5	1·15
H 29. "M.F."	**G.P. 1329**	− 3·5	2·8
H 17. "Sp.B."	**Calf 265**	− 3·	3·
H 49. "T.C."	**Direct**	− 2·	2·9
H 49. "T.C."	**Direct**	− 2·	2·6
H 2. "Sp.A."	**Calf 93**	1·5	2·35
H 23. "J.P."	**Calf 365**	0·5	3·8
H 52. "T.F."	**G.P. 1565**	+ 1·	2·8
H 61. "E.C."	**Direct** (Mesenteric Glands)	+ 1·	2·5
H 56. "F.T."	**G.P. 1682**	+ 1·	2·3
H 17. "Sp.B."	**Rabbit 181**	+ 1·	2·9
B IX.	**Dog 50**	+ 2·	3·6
H 54. "C.W."	**G.P. 1609**	+ 2·	4·0
H 17. "Sp.B."	**Calf 339**	+ 2·5	2·9
H 37. "O.J."	**G.P. 1427**	+ 3·	2·6
H 2. "Sp.A."	**Calf 111**	+ 3·5	3·3
H 19. "S.W."	**Goat 11**	+ 4·5	4·3
H 26. "K.M."	**G.P. 1326**	+ 4·	2·6
H 39. "M.B."	**Direct**	+ 5·	3·2
H 41. "A.S."	**G.P. 1435**	+ 5·	3·5
H 51. "H.M."	**Direct**	+ 5·5	2·8
H 22. "F.W."	**Calf 293**	+ 6·	2·8
H 17. "Sp.B."	**Rabbit 181**	+ 6·	3·4
H 44. "D.C."	**Direct**	+ 7·	3·9
H 27. "B.D."	**G.P. 1216**	+ 7·5	4·0
H 12. "H.N."	**Calf 123**	+ 7·5	3·3
H 57. "B.J."		+13·	2·8

DETAILED DESCRIPTION OF THE EXPERIMENTS.

VIRUS B I.—(Cow 44.)

The culture used for the inoculation was taken from broth and was the sixth generation, the last three of which had been grown on broth.

The broth was fermented and contained 2 per cent. glycerin.

During the first 70 days the inoculated material showed no increase in area but slowly increased in thickness becoming warty and nodular; then a grey film began to grow from the margins; this gradually extended and the whole surface was covered on the 91st day, the growth climbing up the sides of the flask; the pellicle subsequently thickened a little and became greyish white and slightly wrinkled.

Initial titration - - -+12·5
Reaction after 77 days - -+12
(pellicle slowly extending)
Reaction after 91 days - -+ 8
(surface covered)
Reaction after 96 days - -+ 2 or 3
,, ,, 106 ,, - -+ 3
,, ,, 151 ,, - -broth neutral or faintly alkaline.

VIRUS B I.—(Cow 44.)

The above culture was inoculated at the same time on 5 per cent. glycerin broth.

Growth during the first 4 weeks as opaque white nodules with stalactitic down-growths; in the fifth week a grey marginal film developed around the nodules; at the end of the seventh week the whole surface was covered with a pellicle which climbed up the sides of the flask; the growth was grey and showed whitish striae radiating out from a central group of opaque nodules; the pellicle thickened slightly and was finally greyish white and finely wrinkled.

The broth became nearly neutral to phenol-phthalein after the surface was covered; then a development of moulds stopped the experiment.

(This experiment is not included in the table).

VIRUS B I.—(Cow 44.)

The culture used for the inoculation was the twenty-third generation, the last four of which were grown on glycerin broth.

The broth was not fermented and contained 2 per cent. glycerin.

Growth took place from the outset and in a little more than 3 weeks the surface was covered with a thin greyish-white lace-like membrane; during the next fortnight the pellicle became slightly thicker and whiter, some parts being a little thinner than others. The pellicle then broke up into islands and all further growth ceased, many of the islands becoming moist and falling to the bottom of the flask.

Initial reaction - - - - - + 20
Reaction after 21 days - - + 12
,, ,, 39 ,, - + 10
,, ,, 60 ,, - + 10·5
,, ,, 79 ,, - + 11

VIRUS B I.—(Original Material, through Guinea-Pig 14.)

The culture used was the twenty-second generation, the last two of which were grown on broth.

The broth was fermented and contained 5 per cent. glycerin.

Examined 133 days after inoculation the inoculated material was seen to have increased in thickness and area only to a very slight extent; there was an appearance, however, around the margins as if growth might take place. The syphon apparatus was removed and the flask stoppered with a solid rubber cork. A month later the small island had not apparently undergone any change. Examined 9 days later the whole surface was found covered with a thin grey pellicle traversed by wavy white lines radiating out from an opaque central patch. Subsequently the membrane thickened slightly and became greyish white.

Initial reaction - - - - + 12
Reaction after 164 days - - - + 16
,, ,, 191 ,, - - - + 16

VIRUS B II.—(Calf 134.)

The culture used for the inoculation was taken from broth and was the seventh generation, the first six of which were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In ten days the inoculated material had doubled in area; in 39 days there was an irregular greyish white pellicle covering an area equal to that of a two-shilling piece; this slowly increased in area and thickness and in 75 days there was an opaque greyish white patch covering not quite two-thirds of the surface with a grey film spreading out from the margins; the grey film continued to extend but the whole surface was not covered until the 121st day; the filmy growth then sank to the bottom leaving the opaque patch floating.

Initial reaction - - - - - +21
Reaction after 82 days - - - +11·2
,, ,, 121 ,, - - - +10·6

VIRUS B II.—(Calf 134.)

A flask of the same broth, but containing 5 per cent. glycerin, was inoculated at the same time as the above from the same culture.

In 29 days there was a whitish opaque island the size of a sixpenny bit; this slowly increased in thickness, becoming warty on the surface and showing short stalactitic processes on the under surface; around the margins were delicate grey thread-like processes; after 81 days there was very little further increase, but the growth was yellower and more curved. On the 110th day the whole surface was found covered with a thin grey almost transparent pellicle traversed by fine wavy whitish threads radiating outwards from the opaque patch, which was now of a brownish yellow colour thick and warty, and with raised edges.

Initial reaction - - - - - -	+21
Reaction after 81 days - - -	+17
„ „ 110 „ - - -	+ 8
(surface covered)	
„ „ 132 „ - - -	+8·5

VIRUS B II.—(Bull 22.)

The culture used for the inoculation was taken from glycerin milk and was the 21st generation, nine of which had been grown on glycerin media.

The broth was not fermented and contained 2 per cent. glycerin.

Growth was very slow; ten weeks after inoculation there were three grey translucent islands, the largest about the size of a shilling, each showing one or two small opaque white thickenings.

Initial reaction - - - -	+ 17·5
Reaction after 94 days - - -	+ 15·75

(This experiment is not included in the table.)

VIRUS B IV.—(Original Material.)

The culture used was taken from broth and was the tenth generation, the last two of which were grown on broth.

The broth was fermented and contained 2 per cent. glycerin.

Forty-four days after there was a series of small opaque nodules with stalactitic downgrowths; the nodules slowly increased in size and on the 57th day were seen to be forming grey marginal films; on the 69th day the whole surface was nearly covered with a thin greyish translucent film through which curved whitish lines radiated from the opaque nodules; there was no subsequent increase in the thickness of the film.

Initial reaction - - - -	+ 11
Reaction after 77 days - -	+ 4
(surface nearly covered).	
Reaction after 96 days - - -	+ 3·5
„ „ 106 „ - -	+ 2 or 1

VIRUS B IV.—(Original Material.)

The culture used was the 41st generation, the last seven of which had been grown on liquid media.

The broth was unfermented and contained 2 per cent. glycerin.

Growth took place from the outset and in 13 days the whole surface of the broth was covered with a thin uniform grey membrane. At the end of five weeks the membrane was grey translucent finely wrinkled and about the thickness of ordinary tissue paper. Subsequently no further increase in thickness of the pellicle was observed.

Initial reaction - - - - - -	+ 17
Reaction after 21 days - - - -	+ 12·5
„ „ 72 „ - - - -	+ 11

VIRUS B IV.—(Calf 118.)

The culture used for the inoculation was taken from glycerin milk and was the 22nd generation, the last eleven of which had been grown on glycerin media.

The broth was not fermented and contained 2 per cent. glycerin.

Sixteen days after inoculation there was a delicate grey island with whitish thickenings slightly larger than a shilling; this slowly increased and ten weeks after inoculation about a quarter of the surface was covered; the pellicle was grey and translucent and showed opaque white thickenings; there was no further increase in the area of the pellicle and the opaque thickenings acquired a chalky white appearance.

Initial reaction - - - -	+ 17·5
Reaction after 94 days - - -	+ 16·5

(This experiment is not included in the tabular summary of results.)

VIRUS B IV.—(Dog 18.)

The culture used was taken from broth and was the eleventh generation.

The broth was unfermented and contained 2 per cent. glycerin.

Three weeks after inoculation there was an opaque white warty island, measuring 3 by 1½ in. On the fiftieth day two-thirds of the surface was covered with a moderately thick opaque white warty pellicle, whilst on the rest of the surface there were floating some opaque nodules with grey filmy margins. Sixteen days later the broth was found to have become turbid. A smear preparation made from the pellicle showed a few large diplococci scattered about.

Initial reaction - - - - - -	+17·5
Reaction after 50 days - - - -	+24
„ „ 66 „ - - - -	+23·

(This experiment is not included in the tabular summary of results.)

VIRUS B V.—(Heifer 80.)

The culture used was the seventh generation, the last two of which had been grown on a liquid gelatine medium.

The broth was fermented and contained 5 per cent. glycerin.

In 29 days the whole surface was covered with a thin uniform greyish translucent growth; subsequently no change was observed in the appearance of the growth.

Initial reaction	+ 11
Reaction after 29 days	+ 7
,, ,, 48 ,,	+ 8
,, ,, 77 ,,	+ 8·5

VIRUS B V.—(Goat 2.)

The culture used was the seventh generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

In 34 days there was an island the size of a two-shilling piece; in 42 days a third and in 47 days the whole of the surface was covered with a pellicle which subsequently became of a moderate thickness; it was whitish and the surface was moist and reticulated. The initial reaction was + 13 and the final + 5.

VIRUS B V.—(Goat 2.)

The culture used was the tenth generation and was the second broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

In 31 days the whole surface was covered, the growth climbing up the sides of the flask; the pellicle was not uniform, part being opaque white and granular on the surface, the rest thin grey and translucent; later the growth became thicker and more uniform; it was opaque white wrinkled and resembled heavy lace; no increase in thickness was observed after the 64th day; on the 110th day scattered pearly white colonies were seen to have developed on the surface

Initial titration	+21
Reaction after 34 days	+18
,, ,, 49 ,,	+12
,, ,, 64 ,,	+11
,, ,, 81 ,,	+10
,, ,, 110 ,,	+15
,, ,, 139 ,,	+13·5
,, ,, 142 ,,	+10·3

VIRUS B V.—(Goat 2.)

The culture used was the tenth generation and was the second broth subculture.

The broth was fermented and contained 5 per cent. glycerin.

In 31 days there was a thick opaque white pellicle covering not more than a fifth of the surface; this became broken up into islands which increased in area but never coalesced; the islands were opaque white and thick with a pitted surface; not more than half the surface was covered.

Initial reaction	+ 12
Reaction after 34 days	+ 11
,, ,, 81 ,,	+ 6·25
,, ,, 110 ,,	+ 7

VIRUS B VIII.—(Original Material.)

The culture used was the 31st generation, the last two of which had been grown on liquid media.

The broth was unfermented and contained 2 per cent. glycerin.

Seven days later there was a grey patch the size of a shilling with whitish thickenings. This slowly extended and on the 25th day about half the surface was covered with a thin grey pellicle containing irregular opaque whitish patches.

Initial reaction	+ 17
Reaction after 105 days	+ 13·5

VIRUS B IX.—(Original Material, through Guinea-Pig 1069.)

The culture used was taken from the condensation water of a serum tube and was the sixth generation.

The broth was fermented and contained 5 per cent. glycerin.

In 12 days a thin grey membrane speckled with opaque white foci covered from $\frac{1}{6}$ to $\frac{1}{5}$ of the surface; in 18 days the whole surface was covered and in 21 days was climbing up the sides of the flask; the pellicle was thin and grey and was closely speckled with small opaque whitish foci; the growth thickened up to the 33rd day, becoming uniform and greyish white; fissures then appeared in the pellicle the broken edges of which were moist and beginning to sink; two days later the whole of the pellicle had sunk to the bottom of the fluid.

Initial reaction	+ 21
Reaction after 18 days	+ 16·3
,, ,, 33 ,, (surface covered.)	+ 10·2
Reaction after 35 days	+ 11

VIRUS B IX.—(Dog 50.)

The culture used was the 2nd generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Growth was slow. Three weeks after inoculation there was an opaque warty patch, 2½ × 1½ inches; in 46 days about half the surface, and in 58 days the whole surface was covered with a thick opaque white granular pellicle (the growth appeared to be composed entirely of granules each about the size of a pin's head.)

Initial reaction	+ 20
Reaction after 60 days	+ 13·5
,, ,, 81 ,,	+ 16·5
,, ,, 130 ,,	+ 22

VIRUS B IX.—(Dog 60.)

The culture used was the third generation; the first two generations were grown on serum, the third on glycerin gelatine.

The broth was unfermented and contained 2 per cent. glycerin.

Growth took place from the outset and in 18 days the whole surface was covered with a grey translucent membrane mottled with irregular whitish thickenings; the growth climbed up the sides of the flask and already showed a tendency to break up and become moist at the edges; on the twenty-eighth day all the growth had sunk to the bottom of the broth.

Initial reaction	+17.5
Reaction after 19 days	+15
,, ,, 28 ,,	+16

VIRUS B IX.—(Lemur 2.)

The culture used was the fourth generation and was taken from the condensation water pellicle of a serum tube.

The broth was not fermented and contained 2 per cent. glycerin.

Growth took place from the outset and in 21 days a quarter of the surface was covered with a pellicle which was partly opaque white and finely warty, and partly thin grey and almost transparent: 9 days later the rest of the surface was covered with a thin grey membrane which climbed up the sides of the flask; on the thirty-fourth day the opaque patch and on the thirty-eighth day the whole of the pellicle had fallen to the bottom of the flask.

Initial reaction	+17.5
Reaction after 34 days	+14
,, ,, 38 ,,	+11.5

VIRUS B X.—(Original Material, through Guinea-Pig 1070.)

The culture used for the inoculation was taken from broth and was the twenty-fifth generation; the first twenty-four were grown on pure serum.

The broth was not fermented and contained 2 per cent. glycerin.

Growth at first was very slow; at the end of 3 weeks there was a thin grey speckled island less than an inch in diameter. Subsequently growth was more rapid and on the forty-sixth day three-quarters and on the 53rd day the whole of the surface was covered with a thin grey membrane closely studded with raised opaque white colonies. Subsequently the growth became thicker and the colonies more raised.

Initial reaction	+ 20
Reaction after 53 days	+ 13·5
,, ,, 79	+ 11·5
,, ,, 103	+ 11·5

VIRUS B XI.—(Original Material, through Rabbit 66.)

The material for the inoculation was taken from the condensation water of a serum tube and was the fourth generation.

The broth was fermented and contained 5 per cent. glycerin.

In 14 days there was a thin grey patch measuring 1½ + ½ inch; in 35 days the patch measured about 2 + 1 inches and showed opaque white colonies; it slowly increased in area and in 77 days practically covered the whole surface; the pellicle varied in thickness, the greater part being greyish white and studded with opaque white raised colonies, but parts were quite thin and transparent; the surface became completely covered a few days later, but subsequently there was no further change in the growth with the exception of a slight increase in the size of the colonies

Initial reaction	+21
Reaction after 77 days	+ 9·5
,, ,, 96 ,,	+ 8
,, ,, 119 ,,	+10·5

VIRUS B XIII.—(Original Material, through Rabbit 116.)

The culture used for the inoculation was taken from broth and was the eleventh generation; the first ten generations were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 6 days there was a thin grey island the size of a shilling; this increased rapidly in area and in 14 days the whole surface was covered with an extremely thin grey membrane veined with fine greyish-white tortuous lines; subsequently no definite increase in the thickness of the pellicle was observed.

Initial reaction	+10
Reaction after 20 days	+ 4
,, ,, 34 ,,	+ 3
,, ,, 65 ,,	+ 1
,, ,, 99 ,,	+ 3

VIRUS B XIV.—(Original Material.)

The culture used was the twenty-fourth generation, the last two of which had been grown on liquid media.

The broth was unfermented and contained 2 per cent. glycerin.

Ten days after inoculation there was a grey island the size of a shilling with opaque whitish thickenings; this slowly increased in area and on the 59th day a little more than half the surface was covered with an irregular grey patch containing opaque and slightly warty patches. There was no further increase in growth.

Initial reaction - - - +17
Reaction after 108 days - +11 The broth went pink in the cold on the addition of phenol-phthalein.

VIRUS B XV.—(Original Material.)

The culture used was taken from broth and was the fourth generation, the first three of which were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 10 days a thin uniform grey pellicle covered the surface of the broth and climbed up the sides of the flask; the pellicle thickened slightly, then became moist, and at the end of 5 weeks nearly half had fallen to the bottom; the growth that remained on the surface became broken up into islands which increased in area and eventually again covered the whole surface of the broth; the pellicle (incomplete) finally was about the thickness of ordinary tissue paper.

Initial reaction - - - +10
Reaction after 14 days - - - + 6·5
(surface covered)
,, ,, 38 days - - - + 4·5
,, ,, 67 ,, - - - + 6·5
,, ,, 103 ,, - - - + 7

VIRUS B XVIII.—(Original Material.)

The culture used was taken from broth and was the fifth generation, the first four of which had been grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 10 days the surface was covered with an extremely thin grey membrane resembling in patches fine lace; at end of 35 days there was very little change; the pellicle was partly thin uniform and almost transparent and partly greyish with delicate lace-like markings; eventually the pellicle became broken up into islands which, however, showed little tendency to sink.

Initial reaction - +10
Reaction after 14 days - + 5
(surface covered)
,, ,, 38 days - + 2
,, ,, 67 ,, - + 1 Before boiling the broth became pink on the addition of phenol-phthalein.
,, ,, 103 ,, - + 3

VIRUS B XXII.—(Original Material, through G.P. 1508.)

The culture used was the fourth generation and was taken from broth; the first three generations were grown on pure serum.

The broth was unfermented and contained 2 per cent. glycerin.

Growth was very slow; a month after inoculation there was a grey island, ¾ in. in diameter, with opaque white warty thickenings from the under surface of which hung short stalactitic down-growths; the island slowly increased in area and in 12 weeks measured about 2 by 1½ in. Growth then ceased. The patch was irregular in outline, unequal in thickness, moist and very warty on the surface, the thicker parts being yellowish in colour.

Initial reaction - - - - - +17·5
Reaction after 130 days - - - - +16·

(This experiment is not included in the tabular summary of results.)

VIRUS B XXVIII.—(Original Material.)

The culture used was the fourth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

The inoculated material very slowly increased in thickness and area. Four weeks after inoculation there was a thick opaque whitish warty island 1 in. × ½ in. with moist submerged margins. The island became a little larger, measuring on the 86th day about 2½ in. × 1½ in., and then sank.

Initial reaction - - ×17
Reaction after 86 days - ×11.5 The broth went pink on the addition of phenol-phthalein before boiling.

(This experiment is not included in the tabular summary of results.)

VIRUS H 2. "Sp. A." (Heifer 11, through Guinea-Pig 340.)

The material was taken from a liquid gelatine medium which had been sown from broth.

The broth used was fermented and contained 2 per cent. glycerin.

In 5 days there was a small grey island with raised whitish foci on the surface; in 16 days the whole surface was covered with an extremely thin grey film with an irregular whitish meshwork; the growth subsequently showed no obvious increase and finally became broken up into islands.

Initial reaction	+ 10
Reaction after 22 days	+ 6·5
,, ,, 39 ,,	+ 7·7
,, ,, 80 ,,	+ 8
,, ,, 88 ,,	+ 9

VIRUS H 2. "Sp. A." (Calf 83, through Guinea-Pig 618.)

The culture was taken from broth.

The broth used was fermented and contained 2 per cent. glycerin.

Sixty-three days later there were three opaque white warty islands, showing stalactic downgrowths; the largest island measured 1¼ in. by 1 in., the others were quite small; 11 days later the surface was almost completely covered with a delicate grey membrane with fine wavy lines radiating out from the opaque patches; on the hundred-and-fifteenth day there was a thin uniform translucent grey pellicle surrounding three opaque patches which had latterly shown no change in character.

Initial reaction	+ 12
Reaction after 74 days	+ 8·5
,, ,, 115 days	+ 9·5

VIRUS H 2. "Sp. A." (Calf 93, through Guinea-Pig 534.)

The culture used was the twentieth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

A week after inoculation there was a thin greyish island, 2½ in. by 1 in., containing opaque whitish foci.

On the sixteenth day the surface of the broth was covered with a grey pellicle speckled with whitish foci; the growth climbed up the sides of the flask.

Five days later the growth began to break up and sink and on the twenty-eight day most had fallen to the bottom of the flask. Several small islands remained floating; these, aggregated loosely together into a patch about the size of a five-shilling piece, gradually increased in thickness, but not in area, and became opaque and white.

Initial reaction	+ 13
Reaction after 21 days	+ 9
,, ,, 35 ,,	+ 12
,, ,, 112 ,,	+ 11·5

VIRUS H 2. "Sp. A." (Calf 111 through Guinea-Pig 690.)

The culture used for the inoculation was taken from milk and was the nineteenth generation; all the previous cultures had been grown on pure serum.

The broth was not fermented and contained 27 per cent. glycerin.

A fortnight after inoculation the surface was nearly covered with a grey translucent membrane mottled with opaque white patches. On the thirty-fifth day the pellicle was thicker and whiter but not wrinkled; from the under surface large flakes of growth were breaking off and falling to the bottom of the fluid. The pellicle finally became thicker more opaque and wrinkled but not warty.

Initial reaction	+ 20
Reaction after 23 days	+ 19
,, ,, 49 ,,	+ 16·5
,, ,, 76 ,,	+ 22
,, ,, 98 ,,	+ 23·5

VIRUS H 2. "Sp. A." (Calf 111, through Guinea-Pig 690.)

The material for the inoculation was taken from broth and was the sixth generation, the first five of which were grown on serum.

The broth was fermented and contained 5 per cent. glycerin.

Growth was very slow; after 71 days there was an opaque island measuring 3 by 1·5 cm.; this slowly increased in area and in 97 days covered half the surface; on the 113th day the whole surface was covered with a thick creamy white wrinkled pellicle which climbed up the sides of the flask; the growth was not quite equal in thickness to that produced by H. 45. "F.M.," and was not so pigmented.

Initial reaction	+ 15
Reaction after 113 days (surface covered).	+ 7
Reaction after 128 days	+ 10·5
,, ,, 142 ,,	+ 13
,, ,, 160 ,,	+ 16
,, ,, 172 ,,	+ 18

VIRUS H 7. "C.M." (Cow 73, through Guinea-Pig 552.)

The culture used for the inoculation was taken from the surface of glycerin milk and was the twentieth generation.

The broth was not fermented and contained 2 per cent. glycerin.

A fortnight after inoculation nearly half the surface was covered with thin grey-speckled islands; on the eighteenth day the whole surface was covered. About a month after inoculation the growth had attained its maximum development; it was not uniform in thickness, the greater part being greyish-white and semi-opaque, the rest thin grey and translucent. Subsequently the pellicle became moist and broke up into islands, many of which sank; at the end of the experiment there was a large amount of deposit.

(The growth closely resembled that obtained with H 53. D.H.)

Initial reaction	+ 20
Reaction after 28 days	+ 9
,, ,, 56 ,,	+ 9·5
,, ,, 81 ,,	+ 12·7

VIRUS H 7. "C.M." (Cow 73, through Guinea-Pig 552.)

The culture used was the fifteenth generation and was the first broth subculture.

The broth was fermented and contained 5 per cent. glycerin.

Growth was very slow at first; at the end of 30 days there were three small opaque nodules; these gradually increased in thickness and extent, the largest measuring 52 days after inoculation ¾ in. by ¼ in.; they continued to grow and on the sixty-sixth day a fringe of thin grey growth was seen around each of them. Growth was now rapid and 10 days after the membrane was seen to be beginning to form the surface was covered with a thin grey pellicle which climbed up the sides of the flask, and which subsequently thickened slightly and became slightly wrinkled; the opaque islands meanwhile also increased in thickness and area (largest 1¼ in. by ½ in.) and were moist looking and very warty on the surface.

Initial reaction - - - - -	+21
Reaction after 98 days - - -	+ 8·3
,, ,, 129 ,, - - -	+ 9·6

VIRUS H 8. "S.C." (Original Material.)

The culture used was the eleventh generation and was taken from broth.

The broth was fermented and contained 5 per cent. glycerin.

Growth rather slow in the beginning; in 23 days there was a thick white island measuring 1¾ in. by 1 in.; this increased fairly rapidly in size and in 37 days half the surface was covered with a thick opaque white pellicle, one margin of which was submerged, and half with a thin translucent grey membrane. On the fifty-fifth day more of the opaque pellicle was submerged and the thin membrane had become a little thicker; subsequently there was no appreciable change.

Initial reaction - - - -	+11·5
Reaction after 37 days - - -	+ 9·6
,, ,, 55 ,, - - -	+12
,, ,, 92 ,, - - -	+18

VIRUS H 9. "C.T." (Calf 183, through Guinea-Pig 996.)

The culture used for the inoculation was taken from broth and was the fourth generation, the first three of which were grown on pure serum.

The broth was unfermented and contained 5 per cent. glycerin.

Fifteen days later there was a thin grey pellicle covering about a quarter of the surface; in 23 days three-quarters, and in 35 days the whole of the surface was covered, the growth climbing up the sides of the flask; the pellicle was greyish white opaque and wrinkled and of moderate thickness; slight agitation caused numerous flakes of growth to fall to the bottom of the fluid; subsequently no change in the character of the growth was observed.

Initial reaction - - - -	+10·5
Reaction after 15 days - - -	+ 9
,, ,, 23 ,, - - -	+ 8·25
,, ,, 35 ,, - - -	+ 8
,, ,, 48 ,, - - -	+ 6·75
,, ,, 67 ,, - - -	+10
,, ,, 75 ,, - - -	+11
,, ,, 86 ,, - - -	+10·5
,, ,, 98 ,, - - -	+10·5

VIRUS H 10. "B.S." (Heifer 231.)

The culture used for the inoculation was the eighteenth generation and was the first subculture on broth.

The broth was fermented and contained 2 per cent. glycerin.

After 15 days three-quarters of the surface was covered with an extremely delicate grey lace-like growth; in 25 days the whole surface was covered; the pellicle was thin grey and almost transparent and showed slightly thicker greyish white patches; the pellicle subsequently thickened slightly, the transparent parts becoming translucent and showing a network of curved whitish lines, the greyish white patches becoming opaque and eventually slightly yellow.

Initial reaction - - - - -	+13
Reaction after 25 days - - -	+ 6·5
,, ,, 49 ,, - - -	+ 4
,, ,, 80 ,, - - -	+ 4·5
,, ,, 119 ,, - - -	+ 3·75

VIRUS H 11. "E.D." (Calf 221, through Guinea-Pig 918.)

The culture used was the eighth generation and was the first broth culture.

The broth was fermented and contained 5 per cent. glycerin.

In 19 days there was a thick white island 2 in. by 1 in.; 13 days later about a quarter of the surface was covered and at the end of 54 days there was a thick white pellicle over half the surface and over the rest thin grey patches; at the end of 78 days the whole surface was covered partly with a thick white growth and partly with a thinner greyish white membrane, no further change in growth was observed.

Initial reaction - - - - -	+ 11·5
Reaction after 54 days - - -	+ 10·6
,, ,, 78 ,, - - -	+ 11·5
,, ,, 102 ,, - -	+ 18

VIRUS H 13. "A.D." (Calf 301.)

The culture used was taken from broth and was the fifth generation, the first four of which were grown on serum.

The broth was fermented and contained 5 per cent. glycerin.

In 27 days there was a thick opaque pitted pellicle covering half the surface; in 41 days three-quarters and in 55 days the whole surface was covered with a thick creamy white wrinkled pellicle

Initial reaction	+ 15
Reaction after 27 days	+ 8·5

The reaction diminished still further in acidity and then began to get more acid.

Reaction after 104 days	+ 16
,, ,, 109 ,,	+ 20
,, ,, 115 ,,	+ 20

VIRUS H 16. "J.H." (Calf 273.)

The culture used for the inoculation was taken from broth and was the seventh generation, the first six of which were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 3 weeks the whole surface was covered with a greyish white wrinkled pellicle which climbed up the sides of the flask; this slowly increased in thickness and became creamy and more wrinkled.

Initial reaction	+11·5
Reaction after 21 days	+ 5
,, ,, 36 ,,	+ 8
,, ,, 47 ,,	+13
,, ,, 57 ,,	+11
,, ,, 75 ,,	+11·2
,, ,, 89 ,,	+18

At the same time as the 2 per cent. glycerin broth a flask containing 5 per cent. glycerin broth was inoculated.

In 3 weeks there was an irregular pellicle measuring about 1½ by 1 inch; in 27 days about one-fifth and in 36 days four-fifths of the surface was covered; in 57 days the whole surface was covered with a thick creamy growth which was not so wrinkled as on the 2 per cent. broth, and did not climb up the sides of the flask.

Initial titration	+11·5
Reaction after 36 days	+ 7
,, ,, 47 ,,	+13
,, ,, 57 ,,	+10
,, ,, 75 ,,	+12·5
,, ,, 89 ,,	+20

VIRUS H 16. "J.H.' (Bull 423 A.)

The culture used was the eleventh generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

Fifteen days later three small delicate grey islands were seen on the surface; these thickened, became opaque and yellowish white and from their margins an extremely fine almost transparent film grew out, which in 80 days had covered about three-quarters of the surface; eventually the whole surface was covered, the thin film increasing in thickness only very slightly.

Initial reaction	+13
Reaction after 79 days	+9·25
,, ,, 117 ,,	+3·75

VIRUS H 16. "J.H." (Calf 559.)

The culture used for the inoculation was the third generation and was the first broth subculture

The broth was fermented and contained 2 per cent. glycerin.

Twenty-five days after inoculation there was a thin grey lace-like membrane covering between one-sixth and one-fifth of the surface; in 38 days three-fourths and in 42 days the whole of the surface was covered; the pellicle was thin grey and translucent and broken up into islands with a dull surface; subsequently many of the islands thickened, became opaque, whitish and warty on the surface.

Initial reaction	+13
Reaction after 42 days	+ 4·5
,, ,, 66 ,,	+ 4·5
,, ,, 90 ,,	+ 5·25

VIRUS H 17. "Sp. B."—(Calf 265.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

The inoculated material broke up into small fragments which formed the centres of growth. In 9 days there were numerous small delicate islands which increased rapidly in area and covered the surface in 16 days; the pellicle was thin grey and closely interspersed with fine whitish threads; it subsequently increased to a moderate thickness and was moist whitish and slightly wrinkled; the growth was not uniform, two or three quite thin grey patches being seen.

Initial reaction	+10
Reaction after 16 days	+9
,, ,, 34 ,,	+7
,, ,, 57 ,,	+8
,, ,, 98 ,,	+10
,, ,, 110 ,,	+12
,, ,, 125 ,,	+14

VIRUS H 17. "Sp. B."—(Calf 265.)

The culture used for the inoculation was taken from milk and was the seventeenth generation.

The broth was not fermented and contained 2 per cent. glycerin. A month after the inoculation half the surface was covered with a grey translucent membrane mottled with small opaque raised patches. At the end of 6 weeks the whole of the surface was covered with a greyish-white finely wrinkled pellicle showing one or two warty patches; the growth finally broke up into islands.

Initial reaction	+ 20
Reaction after 42 days	+ 14·5
,, ,, 69 ,,	+ 17·5
,, ,, 91 ,,	+ 19

VIRUS H 17. "Sp. B."—(Calf 339, through Guinea-pig 1235.)

The culture used was the seventeenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

At the end of 40 days about half the surface was covered with an opaque greyish white pellicle with a reticulated surface. Finally the whole surface of the broth became covered with a thick opaque white faintly creamy pellicle with a slightly wrinkled very warty surface.

Initial reaction	+ 18
Reaction after 93 days	+ 20·5

VIRUS H 17. "Sp. B."—(Rabbit 181.)

Culture fourth generation from broth.

The culture was used to inoculate two flasks of fermented broth, one containing 2 per cent., the other 5 per cent. glycerin.

On the 2 per cent. glycerin broth in 24 days there was a number of greyish white islands covering about a quarter of the surface; in 40 days the greater part of the surface was covered with opaque milky white islands; these subsequently, beyond a slight increase in thickness, showed no change and never coalesced.

Initial reaction	+ 13
Reaction after 40 days	+ 12
,, ,, 104 ,,	+ 9·5

On the 5 per cent. glycerin the inoculated material showed a slight increase in thickness and area; in 52 days there was a thick white patch, irregular in outline, covering between one-sixth and one-fifth of the surface, and at the end of 82 days about a third of the surface was covered; examined at the end of 102 days a thin grey film was seen to have grown out from the margins; this slowly expanded, but never completely covered the remaining surface.

Initial reaction	+ 13
Reaction after 102 days	+ 7
,, ,, 124 ,,	+ 9
,, ,, 135 ,,	+ 10

VIRUS H 17. "Sp. B."—(Rabbit 181.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

Three weeks after inoculation there was an irregular unequally thick island which covered about a sixth of the surface; the island was for the most part opaque and white, the margins being thin and grey. Growth proceeded very slowly; on the forty-ninth day only about half the surface was covered, and on the eighty-fifth day not more than three-quarters; the whole surface was never completely covered; the pellicle, finally, was moderately thick opaque and stiff and had a wavy or wrinkled surface; the pellicle along the top of the waves, or ridges, was cracked.

Initial reaction	+ 17·5
Reaction after 49 days	+ 17·5
,, ,, 85 days	+ 19·25
,, ,, 109 days	+ 23·5

VIRUS H 17. "Sp. B."—(Rabbit 181.)

The culture was the nineteenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Growth from the outset was very slow; eighteen days after inoculation there was an opaque white island the size of a half-crown piece; this gradually extended and finally the whole surface was covered with a thick wrinkled slightly warty pellicle of a whitish colour with a faint yellow tinge.

Initial reaction	+ 20
Reaction after 71 days	+ 21

VIRUS H 17. "Sp. B." (Calf 555.)

The culture used was the twelfth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

Fourteen days after inoculation the surface of the broth was completely covered with a thin grey membrane which climbed up the sides of the flask: the pellicle was not uniformly thin but showed slightly opaque greyish white patches. There was very little subsequent increase in the thickness of the pellicle which towards the end of the experiment broke up into islands and formed a small amount of deposit at the bottom of the flask.

Initial reaction	+17·5	
Reaction after 14 days	+11	
,, ,, 29 ,,	+ 6	
,, ,, 50 ,,	+ 6	The broth became faintly pink on the addition of phenol phthalein in the cold.
,, ,, 85 ,,	+ 7	

VIRUS H 17. "Sp. B." (Calf 555.)

The culture used was taken from broth and was the fifth generation.

The broth was fermented and contained 2 per cent. glycerin.

Nine days after inoculation there were a few small greyish white lace-like islands; in 23 days about half the surface was covered with small islands, majority greyish white with a glazed surface, but some grey and translucent; in 39 days the whole surface was covered; the pellicle was not uniform and consisted of opaque greyish-white patches joined together by a thin almost transparent membrane; the opaque patches later showed a scaly surface and upturned edges.

Initial reaction	+ 10
Reaction after 39 days	+ 5·25
,, ,, 57 ,,	+ 9
,, ,, 98 ,,	+ 8·5

VIRUS H 17. "Sp. B." (Calf 571.)

The culture used was taken from broth and was the fifth generation.

The broth was fermented and contained 2 per cent glycerin.

Twenty-three days after small very thin greyish lace-like islands were seen scattered over the surface; these increased in area and covered the surface at about the thirty-fifth day; the majority of the islands remained thin and grey, but a few thickened up slightly and became opaque greyish white.

Initial reaction	+10
Reaction after 56 days	+ 7
,, ,, 94 ,,	+ 4·75

VIRUS H 12. "H.N." (Calf 123.)

The culture used was the sixteenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Growth at first was very slow; at the end of the twenty-fourth day there was an opaque white slightly warty patch, the size of a shilling piece, dark-coloured around the margins and on the under surface. Examined on the fifty-second day the whole surface was found covered with a greyish white semi-opaque finely wrinkled pellicle in the midst of which there was a thick opaque dark-coloured warty patch; the pellicle subsequently became thicker, more wrinkled and creamy white in colour. A few days before the termination of the experiment the broth became slightly cloudy; no contaminating organisms were found in microscopical preparations of the pellicle and subcultures from the broth on serum remained sterile.

Initial reaction	+17
Reaction after 54 days (surface covered.)	+20
Reaction after 70 days	+20·7
,, ,, 99 ,, (broth cloudy.)	+24·5

VIRUS H 12. "H.N." (Calf 123.)

The culture used was the sixteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

During the first five weeks there was merely a slight increase in thickness of the inoculated material. On the thirty-ninth day growth was observed around the margins of the small opaque island and 14 days later the whole surface was covered with an extremely thin almost transparent pellicle. The pellicle slowly thickened and became slightly wrinkled but remained translucent and of about the thickness of ordinary tissue paper. A few scattered opaque white colonies developed on the surface and attained the size of pins' heads.

Initial titration	+15
Reaction after 54 days (surface covered.)	+15
Reaction after 70 days	+11·5
,, ,, 100 ,,	+8
,, ,, 126 ,,	+6·5

VIRUS H 14. "F.S." (Calf 125.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

Twenty-two days later the inoculated material had increased in area to that of a shilling piece: 6 days after a small grey island was seen besides the larger one; the two islands increased in area and thickness and on the forty-fifth day began to send out thin grey expansions from the margins; in 9 days the whole surface was nearly covered; the pellicle then was partly composed of thick opaque whitish patches, nodular and irregular on the surface and partly of a delicate grey membrane traversed by slender whitish curved threads; there was no subsequent increase noted.

Initial reaction	+13
Reaction after 59 days	+ 3·5
,, ,, 88 ,,	+ 5·0

VIRUS H 15. "I.W." (Calf 311.)

The culture was the fifth generation and was taken from broth.

The broth was fermented and contained 5 per cent. glycerin.

In 27 days there was an irregular thick pellicle covering about a sixth of the surface; in 39 days three-quarters, and in 53 the whole of the surface was covered with a thick white growth which became very warty on the surface.

Initial reaction	+10
Reaation after 63 days	+ 7
,, ,, 73 ,,	+10·3
,, ,, 82 ,,	+11·3
,, ,, 98 ,,	+18·

VIRUS H 18. "T.T." (Calf 405.)

The culture used was the fourth generation and was taken from broth; the first three generations were grown on serum.

The broth was fermented and contained 5 per cent. glycerin.

Twenty-five days later there was an island measuring 2 cm. by 1 cm.; in 56 days there was a thick opaque creamy pellicle covering between a fifth and a quarter of the surface; in 75 days half the surface was covered, the growth now being very warty; on the eighty-sixth day three-quarters of the surface was covered and a thin grey membrane was spreading out from the margins; on the one hundred and fourth day the whole surface was practically covered; the thin part then thickened up, the pellicle finally being thick wrinkled and warty on the surface.

Initial reaction	+ 15
Reaction after 104 days	+ 17·6
,, ,, 124 ,,	+ 28 broth (acid to litmus.)

VIRUS H 19. "S.W."—(Heifer 239.)

The culture used was the sixteenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

A fortnight after inoculation there was a small opaque island, about the diameter of a sixpenny piece, unequal in thickness and very irregular on the surface. This very slowly increased in area and at the end of three months was about the size of a half-crown piece; it was very thick, opaque and greyish white and had a warty irregular surface. About this time whitish processes began to grow from one margin; these formed a network and when a small patch had formed became detached from the opaque island.

The network expanded very slowly and on the 198th day nearly the whole surface of the broth was covered with a lace-like network composed of moderately thick white trabeculæ; these trabeculæ were very brittle, the slightest jar of the flask causing pieces to break off and sink; eventually the whole surface was covered and the interstices of the network became filled in with an extremely thin grey membrane. In the meantime the original island gradually became moist around the edges and finally sank to the bottom of the fluid.

Initial reaction	+ 17·5
Reaction after 198 days	+ 23·5
,, ,, 203 ,,	+ 23
,, ,, 276 ,,	+ 22

VIRUS H 22. "F.W."—(Calf 293.)

The culture used was taken from broth and was the fifteenth generation, the first fourteen of which had been grown on pure serum.

The broth was not fermented and contained 2 per cent. glycerin.

Twenty-four days later there was an opaque white island measuring 1½ inch by 1 inch; this island slowly expanded and in about sixty days the whole surface was covered; the pellicle was partly thick opaque white and warty, partly thin greyish white and wrinkled; it subsequently increased in thickness and became more wrinkled, the warty part acquiring a creamy tinge.

Initial reaction	+17
Reaction after 55 days	+17·5
,, ,, 70 ,,	+18
,, ,, 97 ,,	+23

VIRUS H 23. "J.P."—(Calf 365.)

The culture used for the inoculation was taken from glycerin milk, and was the tenth generation; eight of the generations were grown on pure serum and one (the ninth) on glycerin broth.

The broth was fermented, and contained 2 per cent. glycerin.

In seventeen days there was a greyish-white island 1 inch in diameter; in twenty-eight days a third of the surface was covered with an opaque whitish granular pellicle, whilst the rest of the surface was incompletely covered with a thin grey membrane; the opaque part of the pellicle increased in thickness and in area, covering eventually about half the surface, and acquired a slight creamy tinge; the grey membrane became translucent, then broke up, parts sinking to the bottom of the fluid.

Initial titration	+14
After 42 days	+ 6·5
,, 66 ,,	+ 9
,, 101 ,,	+14
,, 115 ,,	+13·5

VIRUS H 26. "K.M."—(Original material, through Guinea-Pig 1326.)

The culture used for the inoculation was taken from broth and was the fourteenth generation, the first thirteen of which had been grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

Twenty-four days after inoculation there was a thin, greyish-white island, the size of a threepenny piece, and several small fragments; around the margins of these a delicate transparent growth developed, and on the fifty-second day the whole surface was covered with a thin grey translucent moist pellicle showing a few small opaque whitish patches; the pellicle increased very little in thickness, and became broken up into islands, many of which sank to the bottom of the fluid.

Initial reaction	+ 15
Reaction after 55 days	+ 14·75
,, ,, 74 ,,	+ 9·75
,, ,, 91 ,,	+ 7·5

VIRUS H 26. "K.M."—(Original material, through Guinea Pig 1326.)

This flask was sown from the same broth culture as the above.

The broth was not fermented and contained 2 per cent. glycerin; there was a small amount of muscle sugar present.

At the end of fourteen days there were two irregular opaque warty islands, with thin grey margins, covering about a fifth of the surface. In twenty-eight days five-sixths of the surface and in forty-two days the whole surface was covered; the pellicle was unequal in thickness, the greater part being creamy wrinkled and opaque, the rest greyish-white and translucent. The pellicle subsequently thickened slightly, the more opaque parts becoming distinctly warty.

Initial reaction	+ 17·5
Reaction after 32 days	+ 15·5
,, ,, 44 ,,	+ 14
,, ,, 71 ,,	+ 22
,, ,, 95 ,,	+ 21·5

VIRUS H 27. "B.D."—(Original material, through Guinea-Pig 1216.)

The culture used for the inoculation was the twelfth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

In a fortnight a third, and in a month the whole surface was covered with a greyish translucent finely wrinkled growth containing here and there thicker opaque warty patches. The pellicle increased in thickness, but not uniformly, parts remaining thin grey and translucent. The pellicle finally became broken up into islands and there was a large amount of flaky deposit.

Initial reaction	+ 17·5
Reaction after 29 days	+ 19·5
,, ,, 50 ,,	+ 24
,, ,, 94 ,,	+ 25

VIRUS H 28. "C.L."—(Original material.)

The culture used was the tenth generation and was the first subculture on broth.

The broth was fermented and contained 2 per cent. glycerin.

In 35 days the whole surface was covered with a very delicate almost transparent film, showing two small thicker patches; fine whitish threads developed in the thin membrane, and the two opaque patches increased in thickness, one finally becoming yellowish and slightly wrinkled, the other remaining whitish.

Initial reaction	+ 14
Reaction after 52 days	+ 5·25
,, ,, 89 ,,	+ 1·5

VIRUS H 29. "M.F."—(Original material, through Guinea-Pig 1334.)

The culture used for the inoculation was taken from broth and was the thirteenth generation.

The broth was unfermented and contained 2 per cent. glycerin.

14 days after inoculation there was a whitish island, with thin grey margins, measuring about 2 by 1 in.; this island slowly increased in area and in 56 days covered about half the surface; the greater part was thick, creamy, irregular and warty on the surface; the margins were thin and greyish and traversed by wavy white lines. The thin grey marginal growth continued to extend but did not completely cover the surface until about 90 days after inoculation. The pellicle was finally composed partly of a thick opaque yellow very warty patch and partly of a greyish white translucent membrane traversed by thick yellow curved trabeculae standing up above the surface as prominent ridges.

Initial reaction	+ 17·5
Reaction after 65 days	+ 14
,, ,, 95 ,,	+ 14
,, ,, 109 ,,	+ 14

VIRUS H. 30. "E.M."—(Original material, through Guinea-Pig 1337.)

The culture used for the inoculation was taken from broth and was the ninth generation; the first eight generations were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 6 days there was a grey island about the size of a half-crown piece; this slowly increased in area and on the twenty-eighth day the whole surface was covered with a uniform moderately thick greyish-white wrinkled membrane which climbed up the sides of the flask. Subsequently the growth became thicker, more wrinkled and creamy white.

Initial reaction	+ 13
Reaction after 28 days (surface covered.)	+ 8
Reaction after 47 days	+ 14
,, ,, 60 ,,	+ 17·5
,, ,, 82 ,,	+ 18

VIRUS H. 31. "L.F."—(Original material.)

The culture used for the inoculation was the fourteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

Three weeks after inoculation there was an opaque white wrinkled island, 2 in. by 1 in.; on the thirty-third day half the surface was covered with an opaque, moderately thick white growth, with a honeycombed surface; around the margins of this delicate grey growth appeared, which in a short time broke up and covered the rest of the surface with small islands; subsequently no increase in the thickness or the area of the pellicle was observed.

Initial reaction	+ 14
Reaction after 109 days	+ 6·5
,, ,, 145 ,,	+ 6·5

VIRUS H. 32. "Y.W."—(Original material—Mesenteric Gland.)

The culture used was the sixth generation and was the first broth subculture.

The broth was fermented and contained 5 per cent. glycerin.

In 23 days there was a thin grey layer containing opaque whitish patches covering half the surface; 6 days later the growth had extended over the whole surface; there was very little subsequent thickening of the pellicle.

Initial reaction	+ 13
Reaction after 31 days (surface covered)	+ 4·5
Reaction after 52 days	+ 5·8
,, ,, 98 ,,	+ 7

VIRUS H. 34.—"C.U." (Original material, through Guinea-Pig 236.)

The culture used was the fourteenth generation and was the first broth subculture.

The broth was fermented, and contained 5 per cent. glycerin.

In 8 days the inoculated material had increased to about twice the size; in 19 days there was a thin grey membrane showing a few whitish foci, covering a little over a quarter of the surface; this continued to expand, and in 35 days the whole surface was covered, it was thin greyish and uniform, except for a few small whitish patches; subsequently no further increase in thickness was observed.

Initial reaction - - - -	+11·5
Reaction after 32 days - - -	+ 9·5
(surface covered)	
,, ,, 51 days - - -	+ 9·5
,, ,, 78 ,, - - -	+10
,, ,, 102 ,, - - -	+12

VIRUS H. 34.—"C.U." (Original material, through Guinea-Pig 236.)

The culture used was the tenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

In 17 days the whole surface was covered; the pellicle was partly grey and translucent, with a whitish lace-like network, and partly greyish-white and opaque, with a finely granular surface; on the thirty-fifth day it was moderately thick white uniform and slightly wrinkled; subsequently the pellicle became slightly creamy, but there was no obvious increase in thickness.

Initial titration - - - -	+10
Reaction after 17 days - - -	+ 5·5
(surface covered)	
,, ,, 35 days - -	+ 7·75
,, ,, 74 ,, - -	+ 9·5
,, ,, 95 ,, - -	+ 9·3
,, ,, 101 ,, - - -	+ 8·75

VIRUS H. 36.—"M.D." (Original material.)

The culture used was the seventh generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

During 21 days no increase in the area of the inoculated material was observed; on the thirty-sixth day there was a greyish-white patch, with a warty surface, the size of a shilling; this slowly increased in area and on the seventieth day there was a pellicle covering three-quarters of the surface; the pellicle was partly whitish and opaque and partly grey and translucent; on the seventy-eighth day the whole surface was covered; the growth then became thicker and on the hundred-and-sixth day was thick uniform creamy white and smooth on the surface.

Initial reaction - - - -	+19·5
Reaction after 70 days - - -	+15
,, ,, 106 ,, - - -	+13
,, ,, 117 ,, - - -	+17
,, ,, 138 ,, - - -	+20·5
,, ,, 152 ,, - - -	+27

VIRUS H 37.—"O.J." (Original material, through Guinea-Pig 1427.)

The culture used was taken from broth and was the fifteenth generation, fourteen of which had been grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

During the first 28 days there was no growth; on the forty-third day slight increase in thickness and area of the inoculated material had occurred; on the fifty-sixth day there was a small opaque white island with a delicate, almost transparent margin; fourteen days later the surface was covered with a very thin grey moist translucent pellicle; this thickened slightly and broke up into islands, which showed a marked tendency to become moist at the edges and sink.

Initial reaction - - - - -	+15
Reaction after 67 days - - - -	+12
,, ,, 91 ,, - - - -	+10

VIRUS H. 37. "O.J."—(Original material, through Guinea-Pig 1427.)

This flask was sown from the same broth culture as the above.

The broth was not fermented and contained 2 per cent. glycerin and a small amount of muscle sugar.

In fourteen days there was a creamy yellow warty island covering about one-fifth of the surface. In about 30 days the whole surface was covered; the pellicle was for the most part thick creamy-yellow wrinkled and warty, but parts were quite thin greyish-white and translucent; there was slight subsequent increase, the pellicle remaining unequal in thickness.

Initial reaction - - - - -	+17.5
Reaction after 29 days - - -	+13
,, ,, 44 ,, - - -	+16
,, ,, 71 ,, - - - -	+19
,, ,, 95 ,, - - - -	+20·5

VIRUS H 38. "J.M."—(Original material, through Guinea-Pig 793.)

The culture used for the inoculation was the eighteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

Ten days after inoculation there were two small grey islands, each the size of a threepenny bit and several small fragments; 14 days later the surface was almost covered with an extremely thin film surrounding a few small opaque whitish patches. The pellicle thickened slightly and on the thirty-ninth day was moist, greyish white and translucent (resembling tissue paper). Cracks then appeared, the edges became moist and on the sixty-eighth day most of the growth had sunk to the bottom.

Initial reaction - - - - -	+15
Reaction after 39 days - - -	+10
,, ,, 46 ,, - - -	+ 9
,, ,, 68 - - -	+ 7

VIRUS H. 39. "M.B."—(Original material.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent glycerin.

In 17 days there was a thick milky white island measuring about 2 in. by ½ in.; this slowly increased in area and from the margins a thin grey membrane began to spread; at the end of 45 days the whole surface was covered, a fifth of the pellicle being opaque white and warty, whilst the rest was thin grey and translucent; the pellicle increased to a moderate thickness and was finally uniform greyish white and very wrinkled.

Initial reaction	+17
Reaction after 48 days	+17
,, ,, 63 ,,	+22
,, ,, 92 ,,	+22

VIRUS H 41. "A.S."—(Original material, through Guinea-Pig 1435.)

The culture used was the eleventh generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

At the end of 17 days there was an irregular opaque white island, with thin grey margins, measuring 2in. by 1in.; in 40 days the whole surface was covered; the pellicle was not uniform, being partly thick, opaque and white and partly thin grey and translucent; it subsequently increased in thickness and was finally uniformly thick creamy white and wrinkled.

Initial reaction	+17
Reaction after 39 days	+15·5
,, ,, 61 ,,	+19·5
,, ,, 90 ,,	+22

VIRUS H 42. "M.R."—(Original material, through Guinea-Pig 1447.)

The culture used was taken from broth and was the seventh generation; the first six of which were grown on pure serum.

The broth was fermented and contained 2 per cent. glycerin.

In 10 days there was a greyish patch covering about a third of the surface; in 17 days the whole surface was covered with a uniform greyish white pellicle; this became wrinkled and a little more opaque, but did not climb up the sides of the flask. After the thirty-fifth day no change in the character of the pellicle was observed except that towards the end of the experiment the surface had become moist and shiny.

Initial reaction	+ 14
Reaction after 17 days (surface covered.)	+ 6·5
Reaction after 30 days	+ 9
,, ,, 52 ,,	+ 13
,, ,, 67 ,,	+ 13·75
,, ,, 106 ,,	+ 13

VIRUS H 44. "D.C."—(Original material.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

In three weeks three-quarters of the surface were covered with a pellicle most of which was opaque, white and slightly warty whilst parts were quite thin and grey. In 4 weeks the whole surface was covered and the growth climbed up the sides of the flask. The pellicle was finally thick creamy wrinkled and very warty. After the last titration but one the broth became cloudy; no organisms were found in microscopical preparations.

Initial reaction	+ 17
Reaction after 23 days	+ 21
,, ,, 39 ,,	+ 25
,, ,, 52 ,,	+ 24

VIRUS H 45. "F.M."—(Original material, through Guinea-Pig 1137.)

The culture was taken from broth and was the seventh generation.

Three flasks of fermented broth were sown; two of the flasks contained 2 per cent. and 5 per cent. glycerin respectively; the third was without glycerin.

On the latter no growth was obtained.

On the 2 per cent. glycerin broth during the first 16 days there was only very slight growth; 8 days later there was a pellicle covering a third of the surface, and on the thirty-first day the whole surface was covered with a greyish white uniform wrinkled growth which spread up the sides of the flask; the pellicle subsequently became much thicker and acquired a creamy yellow colour.

Initial reaction	+11
Reaction after 24 days	+10
,, ,, 31 ,,	+ 7
,, ,, 67 ,,	+10·5
,, ,, 77 ,,	+16·5
,, ,, 88 ,,	+20
,, ,, 102 ,,	+18

VIRUS H 45. "F.M." (Original material, through Guinea-Pig 1137.)

On 5 per cent. glycerin broth growth was much slower than on the 2 per cent.. at the end of 57 days there was an island measuring only 2½ in. by 1 in.: the island then began to enlarge, and on the sixty-seventh day four-fifths of the surface was covered; the pellicle was unequally thick, some parts being quite filmy; the whole surface was speedily covered and the pellicle became thick wrinkled and creamy, but did not climb up the sides of the flask.

Initial reaction	+11
Reaction after 67 days	+ 7
,, ,, 77 ,,	+ 7·5
,, ,, 88 ,,	+10
,, ,, 102 ,,	+16
,, ,, 120 ,,	+28

VIRUS H 48. "W.P."—(Original material, through Guinea-Pig 1494.)

The culture used was the fourth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glycerin.

Twenty days later there were three opaque-white warty islands which by the 28th day covered about a sixth of the surface and showed thin grey marginal growth; the latter slowly extended and covered the surface in about 42 days; the complete pellicle consisted of three opaque white warty islands surrounded by a thin grey membrane showing fine whitish threads radiating out from the opaque patches; the whole pellicle thickened, the membranous part becoming opaque, whitish and finely wrinkled.

Initial reaction - - -	+10
Reaction after 35 days - - -	+ 6·75
,, ,, 71 ,, - - -	+ 8
,, ,, 88 ,, - - -	+12
,, ,, 101 ,, - - -	+10
,, ,, 120 ,, - - -	+11

VIRUS H 49. "T.C."—(Original material.)

The culture used was the tenth generation and was the first broth subculture.

The broth was not fermented, and contained 2 per cent. glycerin. A fortnight after inoculation there was a small irregular grey island with opaque whitish thickenings.

On the twenty-eighth day half the surface was covered, the pellicle was opaque whitish and of medium thickness, with parts quite thin and grey.

Growth continued, and appeared to cease about the eighth week; the surface was not completely covered.

The pellicle finally was opaque, rather broken up and of medium, but not uniform thickness; the surface was wavy, moist, and scaly looking.

Initial reaction - - -	+13
Reaction after 36 days - - -	+11
,, ,, 56 ,, - - -	+ 9·5
,, ,, 77 ,, - - -	+10·5
,, ,, 91 ,, - - -	+10·5
,, ,, 105 ,, - - -	+11

VIRUS H 49. "T.C."—(Original material.)

The culture used for the inoculation was the tenth generation, and was the first broth subculture.

The broth was not fermented, and contained 2 per cent. glycerin.

Fourteen days after inoculation there were three or four small grey islands with whitish thickenings. On the twenty-eighth day three-quarters of the surface was covered with a somewhat broken-up pellicle, not uniformly thick; the greater part was opaque and greyish-white, but parts were quite thin and translucent. In five weeks the whole surface was covered, but the growth did not climb up the sides of the flask; the pellicle was broken up, and was wavy and parchment-like; the space between the opaque parts was filled with thin, grey growth. The pellicle showed no further increase in thickness, but opaque raised colonies developed on the surface which attained the diameter of a pin's head

Initial reaction - - -	+13
Reaction after 35 days - - -	+11
,, ,, 63 ,, - - -	+11
,, ,, 112 ,, - - -	+11

VIRUS H 50. "P.H."—(Original material, through Guinea-Pig 1490.)

The culture used was the fifth generation and was the first broth subculture.

The broth was fermented, and contained 2 per cent. glycerin.

In 12 days there were several greyish-white speckled islands; in 20 days the whole surface was covered with a complete white pellicle; it was grey, with an opaque meshwork of whitish lines, mostly radiating out from slightly raised foci; the pellicle increased to a moderate thickness and was creamy-white and very wrinkled.

Initial reaction - - - - -	+10
Reaction after 20 days - - -	+ 6·75
,, ,, 35 ,, - - -	+ 8
,, ,, 71 ,, - - -	+ 8
,, ,, 88 ,, - - -	+11
,, ,, 101 ,, - - -	+10·5
,, ,, 120 ,, - - -	+12

VIRUS H 51. "H.M."—(Original material.)

The culture used was the seventh generation, the last two of which were grown on broth.

The broth was unfermented and contained 2 per cent. glycerin.

Growth was at first rather slow; at the end of 38 days there was a thick warty island, measuring 3 inches by a little over 1 inch in area; growth was then more rapid and on the sixtieth day the whole surface was covered with a thick opaque greyish-white, or slightly creamy pellicle with a honey-combed or pitted surface.

Initial reaction - - - -	+ 17
Reaction after 66 days - -	+ 20·5
,, ,, 74 ,, - -	+ 22·5

VIRUS H 52. "T.F."—(Original material, through Guinea-Pig 1567.)

The culture used was the fourth generation and was taken from broth; the first three generations were grown on pure serum.

The broth was not fermented and contained 2 per cent. glycerin.

In 17 days a fifth, and in thirty days the whole of the surface was covered with a uniform greyish-white pellicle which climbed up the sides of the flask. The pellicle increased to a moderate thickness, becoming wrinkled and slightly creamy; there was no increase after the forty-fifth day.

Initial titration - - - - -	+17
Reaction after 33 days - - -	+16
,, ,, 60 ,, - - -	+20
,, ,, 84 ,, - - -	+18

VIRUS H 53. "D.H."—(Original material, through Guinea-Pig 1482.)

The culture used for the inoculation was taken from the surface of glycerin milk and was the twelfth generation.

The broth was not fermented, and contained 2 per cent glycerin.

A fortnight after the inoculation the whole surface was covered with a thin, translucent, grey pellicle, of about the thickness of tissue paper; it was finely wrinkled and climbed up the sides of the flask. A month after the inoculation the pellicle was found to have become thicker and whiter in patches, the rest of the membrane remaining thin and translucent. Subsequently there was no further growth, and the pellicle broke up into islands, some of which sank; the experiment was terminated on the 56th day on account of a small mould colony appearing in the depth of the broth.

Initial titration - - -	+20
Reaction after 14 days -	+13
,, ,, 28 ,, - -	+ 9·5
,, ,, 56 ,, - -	+ 8·5

(This experiment is not included in the tabular summary of results.)

VIRUS H 54. "C.W."—(Original material, through Guinea-Pig 1609)

The culture used was the sixth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

Growth took place from the outset, but was very slow. At the end of a month about a quarter of the surface was covered with an opaque creamy warty patch. At the end of 6 weeks two-thirds and at the end of 8 weeks practically the whole of the surface was covered. The pellicle was for the most part thick, creamy and warty, but parts, chiefly around the margins, were thinner, greyish white, and more wrinkled.

Initial reaction - - - -	+ 13
Reaction after 56 days - - -	+ 12
,, ,, 77 days - - -	+ 12·5
,, ,, 105 days - - -	+ 15

VIRUS H 55. "R.D."—(Original material—Mesenteric Gland.)

The culture used was the fifth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

Seven days after inoculation there was a small grey island speckled with opaque white foci. This slowly expanded, and in three weeks half the surface was covered. At the end of 5 weeks the whole surface was covered, and the growth had begun to climb up the sides of the flask. In the seventh week there was a moderately thick, greyish-white, slightly wrinkled membrane showing minute raised white foci scattered over the surface; at one part there was a small warty patch. There was no subsequent increase in the thickness of the pellicle, but the surface became studded with raised opaque colonies.

Initial reaction - - - - -	+ 13
Reaction after 29 days - - -	+ 11
,, ,, 49 days - - -	+ 10
,, ,, 98 days - - -	+ 12
,, ,, 191 - - - -	+ 11·5

VIRUS H 55. "R.D."—(Original material—Bronchial Gland.)

The culture used was the fifth generation and was the first broth subculture.

The broth was not fermented and contained 2 per cent. glycerin.

Seven days after inoculation there was an irregular moist whitish island, the size of a shilling. The island slowly increased in area, and at the end of 5 weeks covered about two-thirds of the surface; the growth was moderately thick, opaque, and in parts quite warty. The growth gradually extended, and the surface became almost, though not completely, covered; the pellicle was not uniform in thickness, parts being denser and more warty than others; at the margin there was a small patch, which was quite thin and grey. Subsequently there was a slight general increase in thickness of the pellicle, which acquired a creamy tinge.

Initial reaction - - - - -	+ 13
Reaction after 34 days - - -	+ 11·75
,, ,, 49 days - - -	+ 9·5
,, ,, 77 days - - -	+ 13·5
,, ,, 98 days - - -	+ 13·5
,, ,, 119 days - - -	+ 13·5

VIRUS H 56. "F.T."—(Original material, through Guinea-Pig 1682.)

The culture used was the eleventh generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Thirty-two days after inoculation there was an opaque greyish-white moderately thick pellicle covering nearly the whole surface. The pellicle soon covered the surface and then began to climb up the sides of the flask; the final growth was thick, creamy-yellow and very wrinkled.

Initial reaction - - - - - -	+ 20
Reaction after 85 days - - - -	+ 21

VIRUS H 56. "F.T."—(Original material, through Guinea-Pig 1682.)

This flask was sown at the same time as the above and with the same culture.

The same broth was used but without the addition of glycerin.

Thirty-two days after inoculation there was an irregular island not larger than a two-shilling piece; it was unequal in thickness, the thicker parts having a reticulated surface. There was no further increase in the area of the pellicle.

Initial reaction - -	+ 20	
Reaction after 85 days	+ 12	The broth became pink on the addition of phenol-phthalein in the cold.

VIRUS H 57. 'B.J."

The culture used was the ninth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Forty days after inoculation the whole surface was covered with an opaque, greyish, finely granular pellicle, with a slightly yellow tinge; the pellicle gradually thickened and became creamy in colour and very warty, but not wrinkled.

Initial reaction - - - - - + 17
Reaction after 93 days - - - - + 30

VIRUS H 61. "E.C."—(Original material—Mesenteric Gland.)

The culture used was the fifth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glycerin.

Thirty-two days later about a third of the surface was covered with moderately thick opaque whitish islands, with a reticulated surface. These slowly increased in area but never completely covered the surface.

Initial reaction - - - - - + 20
Reaction after 85 days - - - - + 21

ADDITIONAL OBSERVATIONS ON THE CHANGES IN REACTION PRODUCED IN GLYCERIN BROTH BY HUMAN AND BOVINE TUBERCLE BACILLI.

After the experiments detailed above were completed an additional series of experiments was undertaken with the object of controlling the previous results.

Twelve strains of bovine origin and sixteen strains of human origin, of which five were virulent for the calf and rabbit, have been investigated.

In order to have the experimental conditions in this series perfectly uniform throughout, the same batch of broth was used; chemically pure glycerin was added to it in the proportion of 3 per cent.

The broth proved to be an extremely favourable medium, and all the cultures grew with great vigour, some of the virulent strains producing pellicles which were almost equal to, and in many cases apparently greater in thickness than, those produced by some of the slightly virulent strains.

After sowing, the flasks were not disturbed until at least two months had elapsed; samples were then withdrawn and tested. In four instances the growths produced by bovine bacilli had, before the end of six weeks, sunk to the bottom of the fluid.

In each case the final amount of growth was estimated volumetrically.

The results have been tabulated and arranged in a numerical series beginning with the strain which reduced the acidity to the greatest extent, and ending with the ones which produced the greatest amount of acid.

The series clearly shows that different strains of tubercle bacilli cannot be differentiated into two classes by means of this test; as in the first experiments, the differences between the initial and final reactions form an unbroken series, in which there is no gap which would justify such a distinction.

With only six strains of tubercle bacilli was the reaction of the broth finally more acid than at the start; this increase varied from +0·5 to +8; the strains causing this increase in acidity have only slight virulence for the calf and rabbit.

In all the other cases the final acidity was less than the initial, but in no case did the broth become neutral to phenol-phthalein, the difference between the initial and final reactions varying from 1 to 9·5. Five of these strains have been shown to possess only slight virulence for the calf and rabbit, whilst the rest are highly virulent.

It is therefore not even possible to draw a line at the point where the initial and final reactions are the same, and say that all the strains which reduce the acidity in any degree are virulent, and that all the strains which increase the acidity are slightly virulent.

It may next be enquired how far the change in reaction has accorded with the amount of growth.

An inspection of the column which gives the volumetric estimations shows that the amount of growth produced by the three strains which caused the greatest reduction in acidity is much less than that produced by the three which formed the most acid. But with these the correspondence ceases; in fact the remaining estimations seem to show that the majority of the more virulent bacilli are incapable of producing with the same or an even greater bulk of growth as much acid as the less virulent bacilli.*

The questions therefore arise—do these differences indicate an essential difference in biochemical action, or are they differences in degree only?

In the case of the slightly virulent organism, it seems quite clear that two chemical processes are at work in the broth—one a reduction in acidity which predominates at first, the other a production of acid which becomes more and more marked as the experiment continues, and which can be referred to some splitting up of the glycerin.

The action of the virulent tubercle bacillus is not so readily explained; Theobald Smith is of the opinion that bovine tubercle bacilli, although they utilise glycerin, do not form acid out of it, and are capable, therefore, of producing an alkaline change only in the medium. If this be so, the change in the alkaline direction ought to bear some proportion to the amount of growth, and to be greater when the growth is luxuriant than when it is scanty, as it is when there is no glycerin in the broth, or when glucose is substituted for glycerin. On examining the list it will be noticed that the diminution in acidity brought about by the virulent tubercle bacilli is in all cases comparatively a slight one, and is in many cases less with large amounts of growth than it is with small ones.

It is difficult to explain these results except on the hypothesis that the virulent tubercle bacilli act on, and produce acid out of, the glycerin in the same way as, though to a less marked extent than the slightly virulent bacillus.

The more saprophytic bacilli no doubt from the outset attack and decompose the glycerin with the formation of acid more readily and in greater degree than the less saprophytic organisms, and it is for this reason that the initial diminution in acidity is never great with the slightly virulent bacillus.

Although the majority of the virulent strains have reduced the acidity of the broth to a greater extent than the slightly virulent, yet it is possible to select out of the series virulent strains and slightly virulent strains which have yielded identical results (*e.g.*, Virus B. XI., and H. 16 J.H. Calf 273, H. 54 C.W. and H. 38 J.M.) or strains in which the differences are so slight as to be negligible, (*e.g.*, Virus B. XI. and H. 50 P.H.).

The differences observed, therefore, in the action of different strains of tubercle bacilli on glycerin broth are, in my opinion, differences in degree and not in kind, and are attributable to variations in saprophytic power which have been shown to exist on other media.

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* It must be pointed out, in connection with the amount of growth, that the volumetric estimations have not in all cases given results quite consistent with the naked eye characters of the growth, *e. g.*, Virus B. XIV. described as being thin, grey and translucent yielded a greater volume of growth than H. 52, T.F., which was moderately thick and creamy-white, and other examples could be found.

TABLE V.

SUMMARY OF THE RESULTS OF THE ADDITIONAL EXPERIMENTS WITH GLYCERIN BROTH.

Virus.	Generation.	Duration of Experiment.	Final Reaction.	Difference between Initial and Final Reaction.	Volume of Growth.	Character of Final Growth.
Virus B IX. (direct)	33rd	64 days	+ 5·5	−9·5	2·1 cg.	Thin grey translucent membrane which sank in the third week.
Virus B VIII. (direct)	34th	64 ,,	+ 6·5	−8·5	2·6 ,,	Thin greyish-white membrane which at the end of five weeks had sunk to the bottom of the broth.
Virus B V (G.-P. 711)	25th	64 ,,	+ 7·	−8·	2·4 ,,	Greyish-white finely-wrinkled membrane which began to sink soon after it had covered the surface.
Virus B XIV. (direct)	28th	64 ,,	+ 8·	−7·	3·4 ,,	Thin grey translucent membrane which soon became moist and sank.
Virus B IV. (direct)	47th	64 ,,	+ 9·	−6·	3· ,,	Somewhat opaque greyish white reticulated membrane.
H 49. "T. C." (Calf 787)	12th	71 ,,	+10·	−5·	3·35 ,,	Moist greyish-white somewhat translucent membrane of medium thickness.
Virus B V. (G.-P. 711)	25th	64 ,,	+10·5	−4·5	2·55 ,,	Moist greyish-white pellicle with a somewhat granular surface.
Virus B I. (Cow 44)	36th	84 ,,	+10·5	−4·5	3·3 ,,	Moderately thick opaque whitish pellicle.
H 32. "Y. W." (direct—mesenteric glands)	24th	85 ,,	+10·5	−4·5	3·1 ,,	Opaque white moist pellicle.
Virus B IV. (Calf 118)	32nd	84 ,,	+11·	−4·	3·2 ,,	Opaque white pellicle of moderate thickness with a slightly warty surface.
Virus B II. (Calf 134)	—	67 ,,	+11	−4·	2·7 ,,	Moist opaque white pellicle, not very thick.
H 49. "T. C." (Calf 797)	13th	82 ,,	+11	−4·	2·2 ,,	Greyish translucent slightly wrinkled membrane.
Virus B X. (G.-P. 1070)	30th	64 ,,	+11·5	−3·5	3·0 ,,	Greyish-white slightly wrinkled membrane containing some opaque white patches.
Virus B XI. (Rabbit 66)	22nd	84 ,,	+12·	−3·	3·5 ,,	Moderately thick opaque white moist pellicle.
H 16. "J. H." (Calf 273)	31st	79 ,,	+12·	−3·	3·2 ,,	Greyish somewhat translucent wrinkled membrane with a yellowish tinge.
H 49. "T. C." (direct)	19th	74 ,,	+12·5	−2·5	2·3 ,,	Grey translucent membrane, closely studded with raised opaque white colonies.
Virus B XI. (Rabbit 66)	27th	84 ,,	+13·	−2·	3·3 ,,	Moderately thick opaque white moist pellicle.
H 17. "Sp. B." (Rabbit 181)	22nd	64 ,,	+13·5	−1·5	2·5 ,,	Pellicle partly thin grey translucent and wrinkled, partly opaque and granular.
H 54. "C. W." (G.-P. 1609)	15th	66 ,,	+13·5	−1·5	2·65 ,,	Pellicle composed of small granules which readily separated from each other on shaking the flask.
H 46. "H. W." (G.-P. 1444)	20th	64 ,,	+14·	−1·	2· ,,	Greyish-white somewhat translucent membrane with innumerable raised pearly granules on the surface.
H 50. "P. H." (G.-P. 1490)	20th	64 ,,	+14	−1·	3·2 ,,	Opaque white wrinkled membrane with a slight creamy tinge.
H 38. "J. M." (G.-P. 798)	28th	71 ,,	+14·	−1·	2·75 ,,	Moderately thick opaque white slightly warty membrane.
H 2. "Sp. A." (Calf 111)	26th	68 ,,	+15·5	+0·5	3·7 ,,	Pellicle partly uniform and white, partly creamy wrinkled and warty.
H 52. "T. F." (G.-P. 1567)	25th	71 ,,	+16·	+1·	2·8 ,,	Moderately thick creamy-white membrane.
H 58. "F. G." (direct)	7th	74 ,,	+16·	+1·	2·65 ,,	Opaque white membrane partly creamy and warty.
H 57. "B. J."	10th	71 ,,	+18·2	+3·2	6·35 ,,	Thick creamy wrinkled and warty pellicle.
H 17. "Sp. B." (Calf 265)	24th	66 ,,	+23·	+8·	3·5 ,,	Moderately thick greyish-white membrane, parts of which were warty.
H 45. "F. M." (G.-P. 1137)	30th	85 ,,	+23·	+8·	4· ,,	Thick creamy-white wrinkled pellicle.

THE CHANGES IN REACTION PRODUCED IN GLUCOSE BROTH BY HUMAN AND BOVINE TUBERCLE BACILLI.

Eleven strains of tubercle bacilli, two of bovine origin and nine of human origin, have been tested on broth containing 1 to 2 per cent. glucose; two of the strains of human origin were virulent, six were slightly virulent, and one (H 53 "D.H.") had intermediate virulence for bovines.

The method of testing the change in reaction has been the same as that employed for the glycerin broth.

Glucose is a very satisfactory substitute for glycerin; all the cultures have grown well and the pellicles formed have been similar in character to those on glycerin broth; in the case of H. 13 "A.D." Rat 15, a strain which grows with great difficulty on glycerin media, a very luxuriant growth was obtained, almost equal to the best obtained with slightly virulent bacilli.

Four different samples of broth were used, with an initial reaction varying from +7 to +22.

The results of the tests are given in Table VI.

In all cases the acidity of the broth was gradually diminished, the virulent strains reacting in precisely the same way as the slightly virulent; the greatest diminution in acidity occurred with those strains which produced the most luxuriant growths.

These experiments with glucose broth, therefore, do not afford results which would justify the division of tubercle bacilli into two classes.

TABLE VI.

SUMMARY OF THE RESULTS ON GLUCOSE BROTH.

Virus.	Initial reaction of broth.	Lowest recorded reaction.	Final reaction.	Difference between initial and final reaction.	Character of Pellicle.	Amount of growth in cubic centimetres.
Virus B V. G-P. 711	+7	+ 2·5	+3	-4	Thin grey and translucent	
Virus B XIV. direct	+7	+ 3	+3	-4	Thin grey and translucent	
H. 45. "F.M." G-P. 1137	+7	+ 1	+1	-6	Opaque creamy-white and wrinkled	
H. 43 "F.F." direct	+7	+ 2	+2	-5	Creamy-white pellicle, surface not completely covered	
H. 11. "E.D." Calf 221	+10	+ 5·5	+5·5	-4·5	Partly creamy, partly translucent and greyish white	2·3
H. 34. "C.U." G-P. 236	+10	+ 5	+5	-5	Slightly wrinkled moderately thick	1·9
H. 2. "Sp. A." Calf 83	+12	+ 6	+6	-6	Partly thick and creamy, partly thin and grey	
H. 17 "Sp. B." Calf 265	+12	+ 4·5	+4·5	-7·5	Creamy white and moderately thick	
H. 46. "H.W." G.P. 1444	+22	+13·5	12	-10	Very thick wrinkled and yellow	3·7
H. 13. "A.D." Rat 15	+22	+14	+14	-8	More than half thick wrinkled and creamy; rest thin grey and translucent	3·1
H. 53. "D.H." G-P. 1482	+22	+11	+11	-11	Greyish-white wrinkled membrane with a slight creamy tinge.	3·0

DETAILED DESCRIPTION OF THE EXPERIMENTS WITH GLUCOSE BROTH.

VIRUS H 11. "E.D."—(Calf 221, through Guinea-Pig 918.)

The culture used for the inoculation was the twelfth generation, and was the first broth subculture.

The broth was fermented, and contained 1 per cent. glucose.

Sixteen days after inoculation three-fourths of the surface were covered with a pellicle, unequal in thickness and broken up into islands. About a fortnight later the whole surface was covered; the growth was for the most part opaque, creamy yellow, and very warty; the rest was thinner and greyish white. Subsequently no change in the character of the pellicle was noted.

Initial reaction	- +10	
Reaction after 19 days	- + 6	
,, ,, 32 ,,	- + 7	
,, ,, 70 ,,	- + 5·5	The broth in the cold became deeply pink on the addition of phenol phthalein, the colour being more pronounced than in the case of H 34. C.U.
,, ,, 102 ,,	- + 5·5	

VIRUS H 2. "Sp. A."—(Calf 83, through Guinea-Pig 618.)

The culture used for the inoculation was the first broth subculture.

The broth was fermented, and contained 1 per cent. glucose.

Growth at first was slow; 18 days after inoculation there was an opaque whitish warty nodule, 5 m.m. in diameter; this fourteen days later measured 1 c.m. in diameter, and was thick, opaque and showed stalactitic processes on the under surface.

The island slowly expanded, and on the forty-fifth day a third of the surface was covered with an opaque, creamy-white patch with a honey-combed surface; around the free margin of this patch delicate grey growth began to form; this spread over the surface to some extent, then broke up into small islands; growth then ceased.

Initial reaction	- - +12	
Reaction after 50 days	- + 6	
,, ,, 91 ,,	- + 6·5	The broth gave a deep pink colouration with phenol phthalein in the cold.
,, ,, 99 ,,	- + 6	

VIRUS H 17. "Sp. B."—(Calf 265.)

The culture used was the thirteenth generation and was the first broth subculture.

The broth was fermented and contained 2 per cent. glucose.

In 9 days the whole surface was covered with a thin grey membrane traversed by fine wavy lines. The membrane increased rapidly in thickness and climbed up the sides of the flask. Five weeks after inoculation it was moderately thick, opaque, uniform and wrinkled, and creamy white in colour. There was very little subsequent increase in thickness and finally all the growth sank to the bottom of the fluid.

Initial reaction - - -	- + 12
Reaction after 14 days - -	- + 10
,, ,, 35 ,, - -	- + 8·2
,, ,, 55 ,, - -	- + 7
,, ,, 84 ,, - -	- + 4·5

VIRUS H 34 "C.U."—(Original material, through Guinea-Pig 236.)

The culture used was the tenth generation and was the first broth subculture.

The broth was fermented and contained 1 per cent. glucose.

In a fortnight the whole surface of the broth was covered with a uniform thin greyish-white pellicle speckled with opaque, white foci. The pellicle increased to a moderate thickness, and 5 weeks after inoculation was opaque white, or faintly creamy, and slightly wrinkled.

Later the pellicle broke up into islands and some of the growth sank.

Initial reaction - -	-+ 10	
Reaction after 17 days -	-+ 5·5	
,, ,, 33 ,, -	-+ 6	
,, ,, 72 ,, -	-+ 5·5	The broth before boiling gave a deep pink colour with phenol phthalein.
,, ,, 103 ,, -	-+ 5	

VIRUS B. V.—(Original material, through Guinea-Pig 711.)

The culture used was the eighth generation, the last two of which had been grown on broth.

The broth was fermented and contained 1·25 per cent. glucose.

Growth took place from the outset, and in 16 days the whole surface was covered with a thin grey membrane, traversed by many fine wavy lines. The membrane became finely wrinkled but increased very little in thickness; the wavy lines, however, grew thicker, whiter, and more opaque.

Initial reaction - - -	- + 7
Reaction after 16 days - -	- + 6
,, ,, 32 ,, - -	- + 2·5
,, ,, 78 ,, - -	- + 3

VIRUS B. XIV.—(Original material, through Guinea-Pig 1271.)

The culture used was the fifth generation and was the first broth subculture.

The broth was fermented and contained 1·25 per cent glucose.

In 3 weeks the whole surface was covered with a thin grey uniform lace-like membrane, dotted over the surface of which were numerous small white foci. The membrane thickened slightly, and was finally translucent, finely wrinkled, and dotted on the surface with minute whitish foci.

Initial reaction - - - - -	+ 7
Reaction after 21 days - - -	+ 7
„ „ 42 „ - - -	+ 4·5
„ „ 78 „ - - -	+ 3

H 45. "F.M."—(Original material, through Guinea-Pig 1137.)

The culture used was the eighteenth generation and was the first broth subculture.

The broth was fermented and contained 1·25 per cent. glucose.

Growth took place from the outset, and in a fortnight the whole surface was covered with a thin grey uniform membrane; this rapidly increased in thickness, and at the end of three weeks was opaque, creamy white, and wrinkled.

Initial titration - - - - - -	+7
Reaction after 15 days - - -	+9
„ „ 21 days - - -	+6·5
„ „ 38 days - - -	+5·5
„ „ 62 days - - -	+1
„ „ 79 days - - -	+1

H 43. "F.F."—(Original Material.)

The culture used was the sixth generation and was the first broth subculture.

The broth was fermented, and contained 1·25 per cent. glucose.

Eleven days after inoculation there was a whitish island the size of a two-shilling piece; this gradually extended, and in 8 weeks about three-quarters of the surface was covered. The pellicle was greyish-white and was partly wrinkled, and partly smooth and studded with raised nodules. The pellicle subsequently became thicker and acquired a creamy colour, but the surface was not completely covered; the nodules increased in size, some attaining a diameter of 2 and 3 millimetres.

Initial reaction - - - - - -	+7
Reaction after 53 days - - -	+5·3
„ „ 66 days - - -	+3·5
„ „ 79 days - - -	+2

H 46. "H.W." (Original material, through Guinea-Pig 1444).

The culture used for the inoculation was taken from milk and was the twelfth generation.

The broth was not fermented and contained 2 per cent. glucose.

Three weeks after the inoculation half the surface was covered with a moderately thick greyish-white wrinkled pellicle, with a thicker yellowish patch in the centre; a week later the whole surface was covered and the growth had a slightly creamy tinge. The pellicle was finally thick, very wrinkled, and deep yellow in colour.

Initial reaction - - - - - -	+22
Reaction after 28 days - - -	+19
„ „ 42 days - - -	+13·5
„ „ 70 days - - -	+12·5
„ „ 99 days - - -	+12

H 13. "A.D."—(Rat 15.)

The culture used was the sixteenth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glucose.

Twenty days later there was a small patch consisting of raised opaque yellow nodules. The patch increased slowly in area and the nodules became larger; in the meantime on the sixty-first day a grey marginal film began to form; this gradually extended and at last the whole surface was covered. At the end of the experiment more than half of the pellicle was thick wrinkled and creamy, whilst the rest was thin grey and translucent and showed scattered raised foci.

Initial reaction - - - - - -	+22
Reaction after 69 days - - -	+21
„ „ 154 days - - -	+14

H 53. "D.H." (Original material, through Guinea-Pig 1482).

The culture used was the twelfth generation and was the first broth subculture.

The broth was unfermented and contained 2 per cent. glucose.

Growth was very slow. On the sixty-first day there were three small yellow nodules, one of which was surrounded by a grey film. One hundred and one days after inoculation the whole surface was covered with a grey translucent finely wrinkled membrane showing in the centre a yellow warty patch. The membrane increased in thickness and become more wrinkled, the summits of the wrinkles having a yellowish tinge.

Initial reaction - - - - -	+22
Reaction after 101 days - - -	+16
„ „ 125 days - - -	+11·5
„ „ 139 days - - -	+11

PRELIMINARY NOTE ON THE CHANGES IN REACTION PRODUCED IN GLYCERINATED LITMUS MILK BY HUMAN AND BOVINE TUBERCLE BACILLI.

The method adopted in the foregoing experiments for studying the chemical changes produced in a liquid medium by the tubercle bacillus has the disadvantage that exact information of the variations in reaction is gained only at relatively long intervals.

In order, therefore, to be able to watch the succession of changes in reaction from day to day, milk coloured with litmus was substituted for broth.

Milk was chosen as the basis of the medium, in preference to broth, on account of the readiness with which slight changes in the colour of the litmus can be detected in it. The medium was prepared in the following way: fresh cow's milk was taken and allowed to stand for twenty-four hours, or more, in a cool place to allow the cream to separate; the milk was then syphoned from beneath the cream and coloured with blue litmus solution; after the addition of 2 per cent. glycerin, the fluid was run into six-ounce medicine bottles, about thirty cubic centimetres being placed in each bottle, and fractionally sterilized; at the end of the sterilization the milk was bluish with a faint purplish tinge. After inoculation the bottles were sealed with paraffin.

On this milk medium tubercle bacilli grow very well and form pellicles indistinguishable in character from those produced by the same bacilli on glycerin broth.

The sequence of changes produced in the colour of the medium during the growth of the bacillus is very characteristic. At first as the inoculated material slowly increases in area the milk acquires a deeper blue tint; then, as the pellicle expands and thickens, the original colour of the medium is regained and gradually changed to a bright red; and finally the milk clots.

This type of reaction is exhibited by all the slightly virulent organisms, when luxuriant growths are obtained; occasionally when the growth has failed to cover the surface, or is thinner than usual, the milk has become red, but has remained unclotted.

Virulent tubercle bacilli up to a certain stage bring about exactly the same changes in the colour of the medium; the colour is at first changed into a more intense blue and then gradually to pink or red; none of the virulent tubercle bacilli has, however, clotted the milk, but in the case of H 49 "T.C.," there was towards the end of the experiment a slight deposition of casein.

With some virulent bacilli the deep blue colour produced at the outset has remained permanent; this has occurred when there has been only a slight increase of the inoculated material or when the membrane formed, though complete, has been delicate and transparent.

On many occasions the litmus, after turning pink, has been completely decolourised, the milk becoming perfectly white; when the bottles in which this change has taken place are removed from the incubator and allowed to stand at room temperature, first the pink colour slowly returns and finally the original colour is restored, wholly or partially according to the amount of growth which had been produced on the surface; control bottles of milk placed under the same conditions underwent no change. These phenomena, which appear to depend upon oxygenation, have been observed with slightly virulent bacilli as well as with virulent bacilli, but most frequently with the latter. In no instance when the colour of the milk has been discharged has clotting taken place; the clots so far produced have all been red.

On litmus milk without glycerin in one case a slightly virulent human bacillus grew with moderate luxuriance and the milk became slightly decolourised and acquired a faint pinkish tinge; in all other cases growth has been scanty and the milk has remained very blue.

Further experiments with this medium are being made: *e.g.*, the influence on the reaction of varying percentages of glycerin and of some of the sugars.

The results so far obtained however show that the medium does not differentiate tubercle bacilli into two classes and in this respect they are in agreement with those obtained by the use of glycerin broth.

MODIFICATION EXPERIMENTS WITH TUBERCLE BACILLI OF BOVINE ORIGIN.

Dr. A. STANLEY GRIFFITH and Dr. F. GRIFFITH.

MODIFICATION EXPERIMENTS WITH TUBERCLE BACILLI OF BOVINE ORIGIN.

It has been shown that different strains of bovine bacilli vary within limits in their capacity for growth on media containing glycerin, but it has been impossible to demonstrate any corresponding variation in virulence. Experiments have been carried out to ascertain whether these properties, cultural characters and virulence, are stable or capable of being altered artificially on culture media or by animal passage.

MODIFICATION BY CULTURE.

On pure serum tubercle bacilli of bovine origin grow better in later than in early generations. Prolonged cultivation on this medium does not in our opinion alter the characteristic type of reaction towards media containing glycerin. With viruses of Class I. sub-cultivated on serum for over two years and then grown on solid glycerin media there is the same initial delay and formation of discrete colonies, but the colonies produced are more numerous, and the final amount of growth is generally greater; and this is the case with all viruses, that the amount of growth ultimately produced on all varieties of media, glycerinated or otherwise, accords with the general increase in saprophytic power.

By sub-culture on glycerin media all viruses grow with increased luxuriance on solid glycerin media, and after a number of sub-cultures varying with different strains a type of growth is finally produced which is practically the same for all viruses. This is an opaque warty or slightly wrinkled layer distinctly better than that obtained with the most easy growing viruses of Class III. The change is most marked in those strains which grow originally with the greatest difficulty. These cultures which have been modified by repeated subculture on solid media containing glycerin show no corresponding increase in capacity to grow on glycerin broth. On the other hand by repeated transferences from broth to broth a greater vigour of growth on this medium can be established.

The increased capacity to grow on glycerin media is retained by the cultures after their passage through animals (calf, rabbit, and guinea-pig), and is manifested by the readiness with which they grow in primary culture on glycerin serum and glycerinated potato.

The cultures so recovered show no greater luxuriance as a result of their residence in the animal body, and even after successive passages through animals, the cultures intervening being maintained purely on glycerin media, do not grow better than the same cultures modified by residence on glycerin media alone.

The influence of glycerin produces no diminution in the virulence of the bovine bacilli for rabbits and calves. This has been shown by comparing the virulence of cultures which have been kept on glycerin serum for periods up to nine months with that of the same cultures which have been kept on serum alone.

MODIFICATION BY ANIMAL PASSAGE.

CHIMPANZEE PASSAGES.

Two Viruses, B. IV. and B. IX., are being passed through two series of chimpanzees; these viruses were chosen as representatives of classes of bovine bacilli which showed respectively the greatest and the least reluctance to grow on glycerin media.

Passage with Virus B. IV.

The first animal in this series (No. 8) was fed with 107 c.c. of milk of Cow 172 [Table IV. A.] containing 10,000,000 tubercle bacilli and died 144 days later of broncho-pneumonia of pneumo-coccal origin. The tuberculous disease was limited to the intestines and the nearest glands. Cultures were isolated from a colic and a mesenteric gland.

The second animal of the series (No. 10) was fed with 0·1 mg. of the culture derived from the colic gland of Chimpanzee 8; the culture used was in the fifth generation, 21 days old, and had been in cultivation for a total period of 117 days. This chimpanzee died in 113 days and showed general tuberculosis with extensive ulceration of the intestinal mucous membrane and great enlargement and caseation of the mesenteric glands. Cultures were isolated from a mesenteric gland and from the lung through a guinea-pig.

The third animal of the series (No. 16) was fed with 0·01 mg. of the culture derived from the mesenteric gland of Chimpanzee 10; the culture used was 21 days old, the third generation, and had been in cultivation for a total period of 60 days. This animal died in 91 days of general tuberculosis, the mesenteric and retro-peritoneal glands being much enlarged and extensively caseated. Cultures were isolated from the spleen and a portal gland.

A fourth chimpanzee, No. 22, was inoculated subcutaneously with 0·001 mg. of the culture isolated from the spleen and died in 87 days of general tuberculosis.

The cultures derived from each of the first three animals, namely, from the mesenteric and colic glands of Chimpanzee 8, from the mesenteric glands and lung of Chimpanzee 10, and from the spleen of Chimpanzee 16, have been tested on the differential media employed in the classification of the bovine viruses. All the strains showed cultural characters identical with those of the culture obtained from the original material of Virus B. IV., producing for example discrete colonies on glycerin serum after the initial delay characteristic of the viruses in Class I.

Cultures from each of the three Chimpanzees, 8, 10 and 16, have been inoculated into rabbits. The results show that there has been no alteration in virulence. (*See* Table 1, page 94.)

Passage with Virus B. IX.

The first chimpanzee in this series (No. 14) was fed with 1·1 mg. of culture derived from the original material of Virus B. IX.; the culture used was 20 days old, twentieth generation, and had been in cultivation for a total period of 447 days. This animal died in 63 days of general tuberculosis, the intestines and mesenteric glands being severely affected. Cultures were isolated from a cervical, a mesenteric, and an ileo-colic gland, and there was no change either in cultural characters or virulence for rabbits and guinea-pigs.

The second chimpanzee in the series (No. 18) was fed with 0·01 mg. of the culture derived from the ileo-colic gland of Chimpanzee 14; the culture used was the fourth generation, 22 days old, and had been in cultivation for a total period of 91 days. This culture was primarily raised on glycerin serum, and was grown on glycerin media until the time of its inoculation. The chimpanzee died in 116 days of general tuberculosis, and cultures were isolated from the spleen. The culture shows no change in cultural characters as a result of its passage through the animal body; there is, however, a slightly increased capacity to grow on glycerin media due to its residence on glycerin media before its inoculation. Its virulence for rabbits is unchanged.

CULTURES FROM OTHER CHIMPANZEES.

Cultures have also been recovered from two other chimpanzees; one, No. 4, inoculated subcutaneously with 1 mg. of culture from the original material of Virus B. IV. died in 56 days of general tuberculosis; the other, No. 6 fed for a week with tuberculous milk (Virus B. IV.) and killed 100 days after the experiment began also showed general tuberculosis. The cultures showed no change in cultural characters or in their virulence for rabbits.

BABOON AND MONKEY PASSAGES.

Six passage experiments are being carried out on monkeys and baboons with three viruses, one with Virus B. IV., two with Virus B. I., and three with Virus B. IX.

Cultures of Virus B. I. recovered from two rhesus monkeys, Nos. 80 and 82, which were killed, when dying of general tuberculosis, in 165 and 176 days respectively, showed no alteration in cultural characters or virulence for rabbits.

BABOON PASSAGE.—VIRUS B. I.

The first animal (No. 8) was fed with milk of Cow 64 (Table I. A) containing one million tubercle bacilli. It was killed 97 days later and showed general tuberculosis, the infection spreading from the tonsils. A rabbit inoculated intraperitoneally with an emulsion made from a cervical gland died in 88 days of general tuberculosis.

The second baboon (No. 64) was fed with 0·1 mg. of culture, which had been in cultivation 533 days, derived from the cervical gland of Baboon 8. This animal died in 101 days of severe general tuberculosis, and cultures were obtained from the lung and a cervical gland.

The culture isolated from Baboon 8 showed a distinct increase in luxuriance on the differential media, growing almost as well as the bovine viruses in Class III.

The culture obtained from the lung of Baboon 64 showed a further increase in luxuriance, behaving in the same way as a bovine culture which has been modified by repeated sub-cultivation on glycerin media. Its virulence for rabbits, however, is equal to that of any of the bovine viruses.

BABOON PASSAGE.—VIRUS B. IV.

The first baboon, No. 22, in this series was fed for 30 days with tuberculous milk of Cow 172 (Table IV. A) and died of general tuberculosis 90 days after the experiment began. A culture was isolated from a cervical gland, and after it had been in cultivation for a total period of 292 days, was given by the mouth in a dose of 0·1 mg. to Baboon 62. This baboon died in 136 days of general tuberculosis and cultures were isolated from the spleen and the lung. These strains as well as that from Baboon 22 were identical in every respect with the culture derived from the original material of Virus B. IV., and there was no alteration in virulence as a result of the passage.

It is interesting to note the stability of the cultural characters and virulence of this virus after prolonged cultivation on artificial media and passage through the bodies of chimpanzees and baboons.

RHESUS MONKEY. BABOON PASSAGE.—VIRUS B. I.

A rhesus monkey, No. 76, was fed with 0·01 mg. of culture derived from Cow 44 (Table I. A.) and was killed when dying 228 days later; the post-mortem examination showed general tuberculosis. A culture was isolated from the spleen and this, after it had been in cultivation for a total period of 310 days, was given by the mouth in a dose of 0·1 mg. to Baboon 66, the second animal of the series.

The baboon died in 133 days of general tuberculosis, and cultures were isolated from the spleen and a cervical gland.

The cultures obtained from both animals were unchanged in their characters on artificial media, and in their virulence for rabbits and guinea-pigs.

RHESUS MONKEY. PASSAGE.—VIRUS B. IX.

The first animal of this series (No. 110) fed with 1·0 mg. of culture from the original material of Virus IX., was killed when dying 80 days after inoculation: the post-mortem examination showed general tuberculosis of a severe type. A culture was isolated from an iliac gland and inoculated subcutaneously into two monkeys, No. 114 receiving a dose of 1·0 mg. and No. 116 a dose of 0·01 mg.

Both animals died of acute miliary tuberculosis in 34 and 36 days respectively, and cultures were isolated in each case from an axillary gland.

The culture from Monkey 116 was used to feed Monkeys 130 and 132, the former receiving 0·01 mg., the latter 0·0001 mg. These animals died in 86 and 134 days respectively of general tuberculosis.

The culture isolated from each of the three animals (Nos. 110, 116 and 132) in the passage showed no change in cultural characters or in virulence for rabbits.

MANGABEY MONKEY AND BABOON PASSAGE.

VIRUS B. IX.

A Mangabey monkey was fed with 1·0 mg. of culture derived from the original material of Virus B. IX.; it died in 80 days of general tuberculosis.

A culture was isolated from a lumbar gland, and after it had been in cultivation for a total period of 49 days was used to feed a mandrill baboon.

This animal, No. 68, received a dose of 0·1 mg. and died in 46 days of cysticercosis (taenia saginata). The only visible sign of tuberculosis was an enlarged and caseous retro-pharyngeal gland; a guinea-pig, however, inoculated with an emulsion of the spleen died of general tuberculosis.

A culture was made from the retro-pharyngeal gland and used to feed Monkeys 126 and 128, the former receiving a dose of 0·01 mg., the latter a dose of 0·0001 mg. Monkey 126 died in 120 days of general tuberculosis; Monkey 128 was killed in 169 days, and showed no tuberculosis.

The cultures isolated from each animal in the series showed no change in cultural characters and that from the last animal (No. 126) was virulent for rabbits.

LEMUR PASSAGE.

VIRUS B. IX.

The first lemur, No. 2, was fed with 1·0 mg. of culture from the original material of Virus B. IX., and when killed after 46 days was found to have general tuberculosis. A culture was isolated from a mesenteric gland and inoculated subcutaneously into Lemurs 12 and 10, the former receiving 1·0 mg., the latter 0·1 mg. Both animals died prematurely in 23 and 30 days respectively. In Lemur 12 there was a local lesion, caseation of the nearest glands and early tuberculosis of the spleen.

In Lemur 10 the disease was rather more advanced, a few minute tubercles being visible in the lungs, spleen and liver. The culture obtained from Lemur 2 was unchanged in cultural characters and virulence for rabbits.

EXPERIMENTS WITH DOGS.

Dogs have been inoculated, subcutaneously, intraperitoneally, and by feeding, with the object of testing their susceptibility towards the bovine bacillus. The experiments have shown that the dog possesses a high degree of resistance. In six instances cultures have been recovered and investigated to ascertain whether residence in this refractory species had produced any modification in cultural characters or virulence. Each case will be considered separately.

Case I.

Dog 26 was inoculated intraperitoneally with an emulsion containing 10·15 million bacilli, made from the tuberculous organs of Guinea-Pigs 1148 and 1150. (Milk of Cow 172, Virus B. IV.): it was killed when very ill after 71 days and showed general tuberculosis. A culture obtained from a ventral mediastinal gland was identical with that derived from the original material of Virus B. IV.

Case 2.

Dog 72 was inoculated intraperitoneally with 1·0 mg. of culture obtained from a guinea-pig inoculated with the original material of Virus B. XXII., and was killed, in good health, 156 days later.

The post-mortem examination showed a few yellow foci in the liver, numerous tubercles of a doubtful nature in the kidneys, and several small fibrous tubercles and two or three small grey nodules in the lungs. There was no tuberculosis of the peritoneum and no tubercle bacilli were demonstrated in any of the lesions except in one of the grey nodules in the lungs, from which a culture was isolated.

This culture grows distinctly better than the culture inoculated, and is now classed amongst the most easy-growing bovine viruses; its virulence for rabbits, however, is unchanged.

The culture was inoculated subcutaneously into another dog, No. 126, in a dose of 10 mg. This animal was killed 99 days after inoculation and showed an ulcer at the site of inoculation, slight tuberculosis of the inguinal and bronchial glands, numerous tubercles in the liver and three translucent tubercles in the lungs. A culture isolated from the liver is still under investigation.

Case 3.

Dog 18 was fed with an emulsion of guinea-pig organs containing 10·15 million tubercle bacilli (Virus B. IV.) and showed after 135 days tuberculosis of two mesenteric glands and a few doubtful tubercles in the lung.

The culture isolated from a mesenteric gland grows much more luxuriantly than Virus B. IV. direct (for characters, *see* page 8 of Culture Report), and indeed better than any of the more easy growing bovine viruses. The culture is not virulent for rabbits and a young dog fed with 50 mg. and killed after 77 days showed slight tuberculosis of the pharyngeal, mesenteric and bronchial glands and two tubercles in the lungs.

Case 4.

Dog 50 was fed with 1·0 mg. of culture of Virus B. IX. and was killed 111 days after; the post-mortem examination showed no disease of the alimentary tract or glands in connection with it, but there was tuberculosis of one lobe of the lung. Two guinea-pigs were inoculated from the lung and a culture was isolated from each.

Both these strains grew more luxuriantly than the original culture of Virus B. IX. and even better than that obtained from Dog 18, corresponding in characters with the slightly virulent bacilli of human origin. A calf inoculated subcutaneously with 50 mg. of culture and killed after 91 days showed a local lesion, caseation of the nearest glands and a few tubercles in the lungs and some of the lymphatic glands. A second calf inoculated subcutaneously with an emulsion (containing 83·7 million tubercle bacilli) made from the prescapular gland of the first calf showed, when killed after 64 days, a local lesion only. The results in rabbits also demonstrated the slightly virulent nature of the culture.

Two adult dogs were inoculated each with 10 mg., one subcutaneously, the other by feeding to ascertain the degree of virulence of the culture for dogs. The dogs were killed 99 and 98 days after inoculation respectively. The former showed a small healing ulcer at the seat of inoculation and two grey tubercles in the lung; in the latter there was a single tubercle in the lung and no sign of tuberculosis elsewhere.

Case 5.

Dog 60 was fed with the same dose of the same culture as Dog 50. It was killed 112 days after, and showed tuberculosis limited to the pharyngeal, mesenteric, and ileo-colic glands.

The culture recovered from a mesenteric gland showed no definite increase in luxuriance and was virulent for rabbits.

This culture was obtained in the usual way by scraping the whole surface of a primary tube. In addition a number of single colonies from one primary culture have been separately investigated with regard to their virulence for rabbits, one colony having shown on glycerin agar a distinct increase in luxuriance; the rabbits were inoculated subcutaneously, each with the growth from a single glycerin agar tube in the third generation, and all developed general tuberculosis of the usual type.

A dog, No. 84, was fed with 50 mg. of the culture obtained in the ordinary way; when killed 73 days later it showed a few miliary tubercles in the lungs and no disease elsewhere. A culture isolated direct from one of the tubercles possesses the same cultural characters and virulence as that from the original virus.

A young dog, No. 130, was inoculated subcutaneously with 48 mg. of culture derived from the single colony, No. 3, which had shown a slightly increased power of growth; this animal died in 85 days of acute general tuberculosis, the inoculation having been partly intraperitoneal; a culture isolated from the liver is still under investigation.

Case 6.

Dog 102, an adult animal, was fed with 100 mg. of culture derived from the original material of Virus B. VIII.; it was killed in 72 days and showed one nodule in the lung and no disease elsewhere; a culture isolated direct from the nodule resembled that inoculated.

Additional Experiments.

The results in two cases, Dog 18 and Dog 50, namely, the isolation of cultures entirely different from those inoculated, suggested the possibility that the bovine bacillus could be modified in cultural characters and virulence by residence in the body of the dog. In order to exclude natural tuberculosis as a source of error the method of inserting into the peritoneal cavity a collodion capsule containing culture was adopted.

In the first experiment the culture used was from the original material of Virus B. IV. The Dog No. 54 was killed after 142 days; the collodion part of the capsule was found ruptured in the centre of a tuberculous tumour in the gastro-splenic omentum and the glass part free in the peritoneal cavity. There was tuberculous disease of the peritoneum, spleen, and kidneys.

Cultures were recovered from the glass part of the capsule and from the tumour. Both strains were found to be identical with the original culture, 12 colonies from a primary culture from the capsule, being individually tested on serum and glycerin serum.

The growth from six of the serum tubes containing 32 mg. was used to feed Dog 82, which was killed 91 days later, and found to be free from tuberculosis.

Dog 92 was fed with 25 mg. of the growth from the corresponding glycerin serum tubes after 68 days incubation. This animal was killed after 64 days and showed tuberculosis of one submaxillary gland, and twelve caseous nodules in the lungs. A culture isolated direct from the lung showed no alteration in character, beyond that due to its residence on glycerin media, and no change in virulence.

In the second experiment the capsule contained the culture isolated from Chimpanzee 8 (Virus B. IV.). The Dog, No. 56, was ill and emaciated when killed. The post-mortem examination showed tuberculosis of the peritoneum and miliary tubercles in the lungs, the capsule, as in the previous experiment, being ruptured and surrounded by a tuberculous tumour. Cultures recovered from the tumour, and from the lung through a guinea-pig, showed no change.

The culture from the tumour was inoculated subcutaneously in a dose of 10 mg. into Dog 74. In this animal, killed after 63 days, there was a local lesion, a tuberculous cavity in the region of the axilla and miliary tuberculosis of the lungs and kidneys. Cultures obtained from the cavity and from a bronchial gland through a guinea-pig were identical with that from the original material of Virus B. IV.

The culture isolated from the bronchial gland was used to feed Dog 116, in a dose of 10 mg. ; the dog was killed after 98 days and showed general tuberculosis of a rather more severe type than that so far produced in dogs by feeding. Cultures have been isolated from a mesenteric gland and three separate lung nodules. Those obtained from the mesenteric gland and two of the lung nodules are identical with that from the original material of Virus B. IV. ; the culture from the third lung nodule grows distinctly better and is equal to any in Class III. The virulence of these cultures has not yet been tested.

PIG PASSAGE.

Virus B. I. has been passed through a series of four pigs in succession, all of which developed general tuberculosis. The total period of residence in the animal body was 551 days.

The first animal was fed with tuberculous milk of Cow 40 and an emulsion made from its mesenteric glands was inoculated subcutaneously into two pigs, No. 10 and No. 2.

From the lesions of one of these, No. 12, two more pigs, No. 14 and 16, were inoculated subcutaneously.

The passage was continued with culture obtained from Pig 16; this was inoculated subcutaneously into Pigs 96 and 98 in doses of 0·1 mg. and 1 mg. respectively. Cultures were isolated from all the pigs, and showed in their reactions on the differential media the characters of the original virus.

The virulence of the culture from the last pig of the series was unaltered.

BOVINE PASSAGES.

Three viruses, namely B. I., B. II. and B. IV., have each been passed through a series of bovines, the number of animals being six in the first, three in the second and three in the third series, and the duration of residence 494, 489 and 693 days respectively. There was no evidence of any alteration in virulence, and culture isolated from the different animals in each series were identical with those from the original viruses.

GUINEA-PIG PASSAGE.

Virus B. II. has been passed through a series of 24 guinea-pigs. The first guinea-pig was inoculated with an emulsion from the original material and each of the others with an emulsion of a tuberculous organ of the preceding animal.

The total period of residence in the guinea-pig's body was 3 years 166 days.

Cultures were isolated from three of the guinea-pigs, including the last of the series.

The cultural characters of the three strains were precisely the same as those of other strains of this virus.

This passage experiment shows clearly that residence in the body of the guinea-pig does not alter the characters of the bovine tubercle bacillus.

SUMMARY OF PASSAGE EXPERIMENTS.

Chimpanzees, baboons and monkeys, although peculiarly susceptible to the action of the bovine tubercle bacillus, were chosen for passage experiments on account of their blood relationship to man. In order to approximate as nearly as possible to a natural mode of infection, the bacilli were inoculated by feeding with culture or with milk containing tubercle bacilli. The passages were continued by culture isolated from the preceding animal in order to encourage the more saprophytic bacilli to increase, and with the same object in several cases the cultures were grown on media containing glycerin.

In the chimpanzee passage with Virus B. IV. three animals were used, the total period of residence in the animal body being 348 days and in culture 138 days.

In the chimpanzee passage with Virus B. IX. two animals were used, the total period of residence in the animal body being 179 days and in culture 538 days.

In the baboon and monkey passages the periods during which the bacilli were in the animal body varied from 198 to 361 days.

With one exception the culture recovered from the last animal in each series showed no change in cultural characters and in all instances the virulence for rabbits remained unaltered. The exceptional case occurred in a passage experiment with Virus B. I. through two baboons, the total period of residence of the culture in the animal body being 198 days. The virus primarily used belonged to the most difficult growing class of bovine bacilli, while that recovered from the last animal grew rather better than any of the most easy growing bovine viruses.

The dog exhibits a high degree of resistance to the action of the bovine tubercle bacillus when introduced into the body by subcutaneous inoculation or by feeding. In five instances cultures have been recovered from dogs experimentally inoculated.

Two dogs were fed with Virus B. IV. and Virus B. IX. respectively and were killed after 135 and 111 days ; in each case the culture isolated grew more luxuriantly than any bovine virus and was slightly virulent. Moreover, when reinoculated into other dogs they were found to be incapable of causing more than minimal lesions. Three dogs were inoculated with Viruses B. IV. VIII. and XXII. A culture isolated from the latter killed after 156 days showed increased luxuriance without change in virulence, while in the others there was not the least alteration.

The results in the first two cases may be explained on two hypotheses—either the lesions were not due to the bacilli experimentally introduced, or the bacilli introduced were modified by residence in the animal body.

In order to test the latter hypothesis, Virus B. IV. was passed through two series of dogs and Virus B. IX. through one series, the periods in which the bacilli were in contact with the tissues being 206, 237 and 185 days. The results were negative, the cultures isolated from the last animal in each series showing not the least change in cultural characters or virulence.

The passage experiments with pigs (551 days), oxen (498, 489 and 693 days) and guinea-pigs (1,261 days) produced no change of cultural characters or virulence in the viruses used.

Conclusions.

These passage experiments, so far as they have gone, show that the bovine tubercle bacillus is very retentive of its special cultural characters and high virulence.

The results with dogs are suggestive, and the passage experiments are being continued in order to ascertain whether the dog can modify the bovine tubercle bacillus.

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F. Griffith.

SYNOPSIS OF PASSAGE EXPERIMENTS.

Virus.	Species of Animal.	Number of Animals in the Passage.	Duration of the Passage.	Result of the Passage on	
				Characters of Culture.	Virulence.
B. I. - -	Ox	6	494 days	No change	No change
B. II. - -	Ox	3	489 days	"	"
B. IV. - -	Ox	3	693 days	"	"
B. IV. -	Chimpanzee	3	348 days	No change	No change
B. IX. - -	Chimpanzee	2	179 days	"	"
B. I. - -	Baboon	2	198 days	Grows more luxuriantly.	"
B. I. - -	Rhesus Monkey and Baboon.	2	361 days	No change	"
B. IV. - -	Baboon	2	226 days	"	"
B. IX. - -	Rhesus Monkey	3	250 days	"	"
B. IX. - -	Rhesus Monkey and Baboon.	3	256 days	"	"
B. IX. - -	Lemur	2	76 days	"	"
B. I. - -	Pig	4	551 days	No change	No change
B. II. - -	Guinea-pig	24	1,261 days	No change	No change
B. IV. - -	Dog	2	206 days	No change	No change
B. IV. - -	Dog	3	237 days	"	"
B. IX. - -	Dog	2	185 days	"	"
B. IV. - -	Dog (26)	1	71 days	"	—
B. VIII. -	Dog (102)	1	72 days	"	—
B. XXII. -	Dog (72)	1	156 day	Grows more luxuriantly, Class I. to Class III.	No change
B. IV. - -	Dog (18)	1	135 days	Grow better than any bovine virus.	And are only slightly virulent for calves and rabbits.
B. IX. - -	Dog (50)	1	111 days		

TABLE 1.

CHIMPANZEE PASSAGE.
WITH
VIRUS B. IV.
(July 29, 1903.)

CHIMPANZEE 8.

Fed on October 28, 1904, with 107cc. milk of Cow 172 (Table IV. A.) containing ten million tubercle bacilli.

Died of broncho-pneumonia March 21, 1905, 144 days after.

P.M.—Tuberculosis limited to intestines and nearest glands.

CULTURE
derived from a colic gland.

RABBITS

Inoculated with culture from original material.

Number.	Dose.	Method.	Duration of Life.	Result.
6 rabbits	0·1 mg.	Intrav.	D. 21 days (average)	G.T.
117	0·1 mg.	Intrap.	D. 40 days	G.T.
87	0·1 mg.	Subcut.	D. 105 „	G.T.
88	0·1 mg.	„	D. 72 „	G.T.
133	1·0 mg.	„	D. 112 „	G.T.

CHIMPANZEE 10.

Fed on July 16, 1905, with 0·1 mg. of culture from Chimpanzee 8.

Died, November 6, 1905, 113 days later.

P.M.—General tuberculosis.

CULTURE
derived from a mesenteric gland.

RABBITS

Inoculated Nov. 6, 1906, with culture from Chimpanzee 8.

Number.	Dose.	Method.	Duration of Life.	Result.
320	0·1 mg.	Intrav.	D. 16 days	G.T.
321	1·0 mg.	Intrap.	D. 14 „	G.T.
324	0·1 mg.	Subcut.	D. 64 „	G.T.
322	1·0 mg.	„	D. 53 „	G.T.

CHIMPANZEE 16.

Fed on January 5, 1906, with 0·01 mg. of culture from Chimpanzee 10.

Died, April 6, 1906, 91 days later.

P.M.—General tuberculosis.

CULTURE
derived from spleen.

RABBITS

Inoculated Jan. 5, 1906, with culture from Chimpanzee 10.

Number.	Dose.	Method.	Duration of Life.	Result.
658	0·1 mg.	Intrav.	D. 20 days	G.T.
664	0·01 mg.	Intrap.	D. 41 „	G.T.
662	0·1 mg.	„	D. 32 „	G.T.
660	1·0 mg.	„	D. 18 „	G.T.
663	0·01 mg.	Subcut.	D. 100 „	G.T.
661	0·1 mg.	„	D. 85 „	G.T.
659	1·0 mg.	„	D. 96 „	G.T.

CHIMPANZEE 22.

Inoculated subcutaneously on July 4, 1906, with 0·001 mg. of culture from Chimpanzee 16.

Died, September 29, 1906, 87 days later.

P.M.—General tuberculosis.

CULTURE
derived from bronchial gland.

CHIMPANZEE 24.

Inoculated subcutaneously on November 21, 1906, with 0·00001 mg. of culture from Chimpanzee 22.

RABBITS.

Inoculated Aug. 4, 1906, with culture from Chimpanzee 16.

Number.	Dose.	Method.	Duration of Life.	Result.
948	1·0 mg.	Intrav.	D. 14 days	G.T.
949	0·1 mg.	„	D. 16 „	G.T.
950	1·0 mg.	Intrap.	D. 9 „	G.T.
951	0·1 mg.	„	D. 42 „	G.T.
954	0·001 mg.	„	K. 54 „	G.T.
955	0·001 mg.	„	D. 45 „	G.T.
952	1·0 mg.	Subcut.	K. 54 „	G.T.
953	0·1 mg.	„	K. 54 „	G.T. (Slight)

TABLE 2.

CHIMPANZEE PASSAGE.

VIRUS B. IX.

(June 21st, 1904.)

CHIMPANZEE 14.

Fed on September 11th, 1905, with about 1·1 mg. of culture derived from the original material of Virus B. IX.

Died, November 13th, 1905. 63 days later.

P.M.—General tuberculosis; intestines and mesenteric glands severely affected.

RABBITS

Inoculated with culture from the original material.

Number.	Dose.	Method.	Duration of Life.	Result.
212	0·1 mg.	Intrav.	D. 18 days	G.T.
(4 rabbits)	1·0 mg.	Intrap.	D. 22 days (average).	G.T.
211	0·1 mg.	Intrap.	D. 30 days	G.T.
(3 rabbits)	1·0 mg.	Subcut.	D. 69 days (average).	G.T.

Culture

derived from an ileo-colic gland.

CHIMPANZEE 18.

Fed on February 12th, 1906, with 0·01 mg. of culture from Chimpanzee 14.

Died, June 8th, 1906. 116 days after.

P.M.—General tuberculosis.

RABBITS.

Inoculated February 12, 1906, with culture from Chimpanzee 14.

Number.	Dose.	Method.	Duration of Life.	Result.
742	1·0 mg.	Subcut.	D. 25 days	S.T.
743	0·1 mg.	Subcut.	D. 103 days	G.T.
744	0·01 mg.	Subcut.	D. 132 days	G.T.
745	0·001 mg.	Subcut.	D. 151 days	G.T.

Culture

derived from spleen.

RABBITS.

Inoculated July 14, 1906, with culture from Chimpanzee 18.

Number.	Dose.	Method.	Duration of Life.	Result.
993	1·0 mg.	Intrav.	D. 16 days	G.T.
994	0·1 mg.	Intrav.	D. 18 days	G.T.
996	1·0 mg.	Intrap.	D. 17 days	G.T.
995	0·1 mg.	Intrap.	D. 30 days	G.T.
997	1·0 mg.	Subcut.	K. 44 days	Early G.T.
998	0·1 mg.	Subcut.	K. 44 days	

TABLE 3.

BABOON PASSAGE.

VIRUS B. I.

BABOON 8.

Fed on January 22, 1904, with milk of Cow 64 containing one million tubercle bacilli.

Killed, April 28, 1904, 97 days later.

P.M.—General tuberculosis.

RABBITS

Inoculated with culture obtained from Cows 40 and 44.

Number.	Dose.	Method.	Duration of Life.	Result.
(7 rabbits)	0·1 mg.	Intrav.	22 days (average)	G.T.
137	1·0 mg.	Intrap.	31 days	G.T.
112	0·1 mg.	Intrap.	31 days	G.T.
136	1·0 mg.	Subcut.	82 days	G.T.
3 rabbits	0·1 mg.	Subcut.	95 days (average)	G.T.

CULTURE
derived from cervical gland

RABBIT.

Inoculated with emulsion from cervical gland.

Number.	Dose.	Method.	Duration of Life.	Result.
48	not estimated.	Intrap.	D. 88 days	G.T.

BABOON 64.

Fed on October 13, 1905, with 0·1 mg. of culture from Baboon 8.

Died, January 22, 1906, 101 days later.

P.M.—General tuberculosis.

CULTURE
derived from lung

RABBITS.

Inoculated on May 1, 1906, with culture from Baboon 64.

Number.	Dose.	Method.	Duration of Life.	Result.
857	0·1 mg.	Intrav.	D. 21 days	G.T.
858	1·0 mg.	Intrap.	D. 15 days	G.T.
859	0·1 mg.	Intrap.	D. 16 days	G.T.
860	1·0 mg.	Subcut.	K. 91 days (Dying)	G.T.

TABLE 4.

BABOON PASSAGE.

VIRUS B. IV.

(July 29, 1903.)

BABOON 22.

Fed with tuberculous milk of Cow 172 from September 9, 1904, to October 4, 1904.

Died December 8, 1904, 90 days after the experiment began.

P.M.—General tuberculosis.

RABBITS.

For rabbits inoculated with culture from the original material *see* Table 1.

CULTURE

derived from a cervical gland.

BABOON 62.

Fed on September 26, 1905, with 0·1 mg. of culture from Baboon 22.

Died, February, 1906, 136 days later.

P.M.—General tuberculosis.

CULTURE

derived from the lung.

RABBITS.

Inoculated on May 11, 1906, with culture from Baboon 62.

Number.	Dose.	Method.	Duration of Life.	Result.
874	0·1 mg.	Intrav.	D. 19 days	G.T.
873	0·1 mg.	Intrap.	D. 16 days	G.T.
872	1·0 mg.	Subcut.	K. 108 days	G.T. (slight)

TABLE 5.

RHESUS MONKEY AND BABOON PASSAGE.

VIRUS B. I.

MONKEY 76.

Fed on April 7, 1904, with 0·01 mg. of culture obtained from the supramammary lymphatic gland of Cow 44.

Killed, when dying, November 21, 1905, 228 days later.

P.M.—General tuberculosis.

RABBITS.

For rabbits inoculated with bovine strains of this virus *see* Table 4.

Culture
obtained from the spleen.

BABOON 66.

Fed on September 27, 1905, with 0·1 mg. of culture from Monkey 76.

Died, February 7, 1906, 133 days after.

P.M.—General tuberculosis.

RABBITS.

Inoculated on February 20, 1905, with culture from Monkey 76.

Number.	Dose.	Method.	Duration of Life.	Result.
189	0·1 mg.	Intrav.	D. 24 days	G.T.
192	1·0 mg.	„	D. 16 „	G.T.
190	1·0 mg.	Intrap.	D. 23 „	G.T.
191	1·0 mg.	„	D. 32 „	G.T.
193	1·0 mg.	Subcut.	D. 47 „	G.T.
194	1·0 mg.	„	D. 63 „	G.T.

Culture
derived from spleen.

RABBITS.

Inoculated on July 6, 1906, with culture from Baboon 66.

Number.	Dose.	Method.	Duration of Life.	Result.
984	1·0 mg.	Intrav.	D. 14 days	G.T.
985	0·1 mg.	„	D. 26 „	G.T.
987	1·0 mg.	Intrap.	D. 12 „	G.T.
986	0·1 mg.	„	D. 1 day	Cocci-diosis.

TABLE 6.

RHESUS MONKEY PASSAGE.

VIRUS B. IX.

(June 21, 1904.)

MONKEY 110.

Fed on July 10, 1905, with 1·0 mg. of culture derived from the original material of Virus B IX.

Killed, when dying, September 28, 1905, 80 days later.

P.M.—General tuberculosis.

RABBITS.

For rabbits inoculated from original material, see Table 2.

CULTURE
derived from an iliac gland.

MONKEY 116.

Inoculated subcutaneously on November 22, 1905, with 1·0 mg. of culture from Monkey 110.

Died, December 28, 1905, 36 days later.

P.M.—Miliary tuberculosis.

MONKEY 114.

Inoculated subcutaneously on November 22, 1905, with 0·01 mg. of culture from Monkey 110.

Died, December 26, 1905, 34 days later.

P.M.—Miliary tuberculosis.

RABBITS.

Inoculated with culture from Monkey 110.

Number.	Dose.	Method.	Duration of Life.	Result.
521	1·0 mg.	Intrap.	D. 22 days	G.T.
522	0·01 mg.	Intrap.	D. 37 „	G.T.

CULTURE
derived from an axillary gland.

MONKEY 130.

Fed on January 24, 1906, with 0·01 mg. of culture from Monkey 116.

Killed when ill, April 20, 1906, 86 days later.

P.M.—General tuberculosis.

MONKEY 132.

Fed on January 24, 1906, with 0·0001 mg. of culture from Monkey 116.

Died, June 7, 1906, 134 days later.

P.M.—General tuberculosis.

RABBITS.

Inoculated on January 24, 1906, with culture from Monkey 116.

Number.	Dose.	Method.	Duration of Life.	Result.
691	1·0 mg.	Subcut.	D. 100 days	G.T.
692	0·01 mg.	„	D. 20 „	S.T.
593	0·0001 mg.	„	D. 100 „	G.T.

MONKEY 152.

Inoculated subcutaneously on June 7, 1906, with a small quantity of emulsion made from the spleen of Monkey 132.

Died, September 26, 1906, 111 days after inoculation.

P.M.—General tuberculosis.

RABBIT.

Inoculated intraperitoneally with an emulsion made from a splenic lymphatic gland.

Number.	Duration of Life.	Result.
1039	D. 75 days	G.T.

RABBITS.

Inoculated on July 27, 1906, with culture derived from the bronchial gland of Monkey 132.

Number.	Dose.	Method.	Duration of Life.	Result.
1022	1·0 mg.	Intrav.	D. 5 days	—
1019	0·1 mg.	Intrav.	D. 16 days	G.T.
1020	0·1 mg.	Intrav.	D. 21 days	G.T.
1021	1·0 mg.	Intrap.	D. 23 days	G T.
1023	0·1 mg.	Intrap.	D. 5 days	—

TABLE 7.

MONKEY AND BABOON PASSAGE.

VIRUS B. IX.

(June 21, 1904.)

MANGABEY MONKEY 4.

Fed on June 2, 1905, with 1·0 mg. of culture derived from the original material of Virus IX.

Killed, August 25, 1905, 84 days later.

P.M.—General tuberculosis.

RABBITS.

For rabbits inoculated with culture from the original material *see* Table 2.

CULTURE
derived from a lumbar gland.

MANDRILL BABOON 68.

Fed on October 13, 1905, with 0·1 mg. of culture from Mangabey 4.

Died, November 28, 1905, 46 days later.

P.M.—Tuberculosis limited to a retro-pharyngeal gland.

CULTURE
derived from the pharyngeal gland.

MONKEY 126.

Fed on January 24, 1906, with 0·01 mg. of culture from Baboon 68.

Died, May 24, 1906, 126 days later.

P.M.—General tuberculosis.

MONKEY 128.

Fed on January 24, 1906, with 0·0001 mg. of culture from Baboon 68.

Killed, July 12, 1906, 169 days later.

P.M.—No tuberculosis.

RABBITS.

Inoculated on January 24, 1906, with culture from Baboon 68.

Number.	Dose.	Method.	Duration of Life.	Result.
688	1·0 mg.	Subcut.	D. 77 days	G.T.
689	0·01 mg.	„	D. 129 days	G.T.
690	0·0001 mg.	„	K. 187 days	G.T.

CULTURE.
derived from spleen.

RABBITS.

Inoculated on July 27, 1906, with culture from Monkey 126.

Number.	Dose.	Method.	Duration of Life.	Result.
1016	1·0 mg.	Intrav.	D. 15 days	G.T.
1015	0·1 mg.	Intrav.	D. 19 days	G.T.
1018	0·1 mg.	Intrav.	D. 25 days	G.T.
1014	1·0 mg.	Intrap.	D. 5 days	—
1017	0·1 mg.	Intrap.	D. 28 days	G.T.

TABLE 8.

LEMUR PASSAGE.

VIRUS B. IX.

(June 27, 1904.)

LEMUR 2.

Fed June 2, 1905, with 1·0 mg. of culture derived from the original material of Virus B. IX.

Killed, July 18, 1905, 46 days after.

P.M.—General tuberculosis.

RABBITS.

For rabbits inoculated with culture from original material, *see* Table 2.

CULTURE
derived from a mesenteric gland.

LEMUR 12.

Inoculated subcutaneously on November 24, 1905, with 1·0 mg. of culture from Lemur 2.

Killed, dying, December 17, 1905, 23 days after.

P. M. — Local abscess, caseation of nearest glands and early tuberculosis of the spleen.

LEMUR 10.

Inoculated subcutaneously on November 24, 1905, with 0·01 mg. of culture from Lemur 2.

Died, December 24, 1905, 30 days after.

P. M. — Local lesion, caseation of nearest glands, a few tubercles in spleen, liver, and lungs.

RABBITS.

Number.	Dose.	Method.	Duration of Life.	Result.
535	1·0 mg.	Intrav.	D. 14 days	G.T.
536	0·1 mg.	„	D. 21 „	G.T.
537	1·0 mg.	Intrap.	D. 18 „	G.T.

TABLE 9.

DOG PASSAGE.

VIRUS B. IV.

(July 29, 1903.)

DOG 54.

A collodion capsule containing an emulsion of culture derived from the original material of Virus B. IV. was inserted into the peritoneal cavity on May 20, 1905.

The dog was killed on October 9, 1905, 142 days later.

P.M.—The capsule was found ruptured and surrounded by a tumour, and there was tuberculosis of the peritoneum, spleen and kidneys.

CULTURE
raised from the interior of the capsule.

DOG 92.

Fed on January 22, 1906, with 25 mg. of culture from Dog 54.

(The culture used was a glycerine serum culture and was 68 days old.)

Killed, March 27, 1906, 64 days after.

P.M.—Tuberculosis of one submaxillary gland: about 12 caseous nodules in the lung.

CULTURE
derived from the lung.

RABBITS.

Inoculated on July 18, 1906.

Number.	Dose.	Method.	Duration of Life.	Result.
1009	1·0 mg.	Intrav.	D. 14 days	G.T.
1010	0·1 mg.	Intrav.	D. 21 days	G.T.
1013	1·0 mg.	Intrap.	D. 20 days	G.T.
1011	0·1 mg.	Intrap.	D. 26 days	G.T.
1012	1·0 mg.	Subcut.	K. 40 days	G.T. (slight)

DOG 82.

Fed on December 15, 1906, with 32 mg. of culture from Dog 54.

(The culture used was a serum culture and was 30 days old.)

Killed, March 16, 1906, 91 days after.

P.M.—There were some doubtful tubercles in the kidneys and one doubtful nodule in the lung (not apparently tuberculous).

TABLE 10.

DOG PASSAGE.

VIRUS B. IV.

DOG 56.

A collodion capsule containing culture from Chimpanzee 8 was inserted in the peritoneal cavity on June 8, 1905.

The dog was killed on August 23, 1905, 76 days after.

P.M.—The capsule was found ruptured and surrounded by a tumour; there was peritoneal tuberculosis and miliary tuberculosis of the lungs.

Culture
obtained from the tumour.

DOG 74.

Inoculated subcutaneously on October 13, 1905, with 10 mg. of culture from Dog 56.

Killed, December 15, 1905, 63 days later.

P.M.—Local lesion; fairly numerous grey miliary tubercles in the lungs; a few tubercles in the kidneys.

Culture
derived from a bronchial gland (normal to the eye) through Guinea-pig 1808, January 26, 1906.

DOG 116.

Fed on March 29, 1906, with 10 mg. of culture from Dog 74.

Killed, July 5, 1906, 98 days later.

P.M.—Tuberculosis of pharyngeal, cervical and mesenteric glands, liver, lungs and bronchial glands.

RABBIT.

Inoculated with emulsion of lung of Dog 116 (very small dose).

Number.	Method.	Duration of Life.	Result.
971	Intrap.	K. 52 days	Very slight tub'rc'los's

TABLE II.

DOG PASSAGE.

VIRUS B. IV.

(July 29, 1903.)

DOG 18.

Fed on August 23, 1904, with emulsion made from organs of Guinea-pigs 1148 and 1150, inoculated with milk of Cow 164.

Dose : 10·15 million tubercle bacilli.

Killed, January 5, 1905. 135 days later.

P.M.—Tuberculosis of two mesenteric glands and a few doubtful tubercles in the lungs.

RABBITS.

For rabbits inoculated with culture from original material *see* Table I.

CULTURE
derived from a mesenteric gland.

DOG 132.

Fed on June 1, 1906, with 50 mg. of culture derived from Dog 18.

Killed, August 17, 1906. 77 days later.

P.M.—Caseous focus in a pharyngeal gland; caseous patches and foci in the mesenteric glands; two tubercles in the lungs and a caseous focus in each bronchial gland.

RABBITS.

Inoculated with culture from Dog 18.

Number.	Dose.	Method.	Duration of Life.	Result.
410	0·1 mg.	Intrav.	K. 97 days	A few T.'s in lungs and kidneys.
890	10· mg.	Intrap.	K. 83 days	A few T.'s on peritoneum, in lungs and kidneys.
409	1·0 mg.	Intrap.	K. 97 days	A few T.'s on peritoneum, in lungs and kidneys.
411	0·1 mg.	Intrap.	D. 68 days	A few T.'s on peritoneum and in lungs.
408	1·0 mg.	Subcut.	K. 97 days	Local lesion, 2 nodules in lung.
412	0·1 mg.	Subcut.	K. 97 days	Ulcerated local lesion, 1 nodule in lung.

TABLE 12.

DOG PASSAGE.

VIRUS B. IX.
(June 21, 1904.)

DOG 60.

Fed on July 10, 1905, with 1·0 mg. of culture derived from the original material. Killed, October 30th, 112 days after.
P.M.—Retrogressive tuberculosis of pharyngeal, mesenteric and ileo-colic glands.

CULTURE
derived from a mesenteric gland.

DOG 84.

Fed on January 2, 1906, with 50 mg. of culture originating from a number of primary colonies.

Killed, March 16, 1906, 73 days later.

P.M.—There were a few miliary tubercles in the lungs, and no disease elsewhere.

CULTURE
derived from the lung.

RABBITS.

Inoculated on July 17, 1906, with culture from Dog 84

Number.	Dose.	Method.	Duration of Life.	Result.
1003	1·0 mg.	Intrav.	D. 17 days	G.T.
1000	0·1 mg.	„	D. 23 „	G.T.
1001	1·0 mg.	Intrap.	D. 21 „	G.T.
1002	0·1 mg.	„	D. 32 „	G.T.
999	1·0 mg.	Subcut.	K. 111 „	G.T. (slight).

RABBITS.

Inoculated with culture originating from a number of primary colonies.

Number.	Dose.	Method.	Duration of Life.	Result.
637	0·1 mg.	Intrav.	D. 28 days	G.T.
639	1·0 mg.	Intrap.	D. 28 „	G.T.
638	0·1 mg.	„	D. 25 „	G.T.
640	1·0 mg.	Subcut.	D. 161 „	G.T.
641	0·1 mg.	„	D. 78 „	G.T.

RABBITS.

Inoculated with cultures originating from single primary colonies. Dose in each case=the growth from one glycerin agar tube.

Number of Rabbit.	Number of Colony.	Method.	Duration of Life.	Result.
729	1	Subcut.	D. 90 days	G.T.
730	2	„	K. 105 „	G.T.
731	3	„	K. 90 „	G.T.
732	4	„	D. 88 „	G.T.
733	5	„	D. 75 „	G.T.
734	6	„	D. 71 „	G.T.
735	7	„	D. 93 „	G.T.
736	8	„	K. 105 „	G.T.
737	9	„	K. 105 „	G.T.

DOG 130.

Inoculated subcutaneously and intraperitoneally on May 17, 1906, with 48 mg. of culture originating from a single primary colony.

Died, August, 1906, 85 days after inoculation.

P.M.—General tuberculosis.

RABBITS.

Inoculated intraperitoneally with an emulsion from the liver.

Number.	Duration of Life.	Result.
1029	K. 55 days.	G T.
1030	D 14 days	S.T.

TABLE 13.

DOG PASSAGE.

VIRUS B. IX.

(June 21, 1904.)

DOG 50.

Fed on June 2, 1905, with 1·0 mg. of culture derived from the original material of Virus B IX.

Killed, September 21, 1905, 111 days later.

P.M.—Tuberculosis of one lobe of the lung, and (?) of the kidneys.

RABBITS.

For rabbits inoculated with culture from the original material, *see* Table 2.

Lung

GUINEA-PIG 1707.

Inoculated September 21, 1905.

Died October 21, 1905 (30 days).

P.M.—General tuberculosis.

GUINEA-PIG 1708.

Inoculated September 21, 1905.

Died October 18, 1905 (27 days).

P.M.—General tuberculosis.

Culture

7th generation. 20 days old.

DOG 108.

Fed on March 21, 1906, with 10 mg. of culture.

Killed, June 27, 1906, 98 days after.

P.M.—One miliary tubercle in the lungs; no sign of tuberculosis elsewhere.

DOG 110.

Inoculated subcutaneously on March 21, 1906, with 10 mg. of culture.

Killed, June 28, 1906, 99 days after inoculation.

P.M.—Small healing ulcer; two grey tubercles in the lungs.

Culture

3rd generation, 20 days old.

CALF 374.

Inoculated subcutaneously on January 2, 1906, with 50 mg. of culture.

Killed April 3, 1906, 91 days later.

P.M.—Caseous local lesion; caseation of nearest glands; a few tubercles in the portal, thoracic, coeliac and ileo-colic glands and the lungs.

CALF 394.

Inoculated subcutaneously on April 3, 1906, with an emulsion from the prescapular gland of Calf 374.

Number of tubercle bacilli.

Killed, June 6, 1906, 64 days after inoculation.

P.M.—Local lesion only.

RABBITS.

Number of Rabbit.	Dose.	Method.	Duration of Life.	Result.
634	0·1 mg.	Intrav.	K. 91 days	T. of lungs, kidneys and muscles.
633	1·0 mg.	„	K. 91 „	G.T.
636	0·1 mg.	Intrap.	D. 68 „	T. of peritoneum and lungs.
632	1·0 mg.	„	D. 81 „	G.T.
635	1·0 mg.	Subcut.	K. 91 „	Slight tuberculosis.

TABLE 14.

DOG PASSAGE.

VIRUS B. XXII.

(April 18, 1905.)

DOG 72.

Inoculated intraperitoneally on September 4, 1905, with 1·0 mg. of culture derived from the original material through Guinea-pig 1509.

Killed, February 7, 1906, 156 days later.

P.M.—There were a few nodules and fibrous tubercles in the lungs, a few yellow foci in the liver and some doubtful tubercles in the kidneys.

RABBITS.

Inoculated with culture from the original material through Guinea-pig 1509.

Number.	Dose.	Method.	Duration of Life.	Result.
419	0·1 mg.	Intrav.	D. 22 days	G.T.
422	1·0 mg.	Intrap.	D. 21 „	G.T.
420	0·1 mg.	Intrap.	D. 28 „	G.T.
418	1·0 mg.	Subcut.	D. 79 „	G.T.
421	0·1 mg.	Subcut.	D. 113 „	G.T.

CULTURE
derived from a lung nodule.

DOG 126.

Inoculated subcutaneously on May 9, 1906, with 10·0 mg. of culture from Dog 72.

Killed, August 16, 1906, 99 days after inoculation.

P.M.—Local lesion; slight tuberculosis of nearest glands; three tubercles in the lungs; bronchial glands slightly affected; liver contained numerous miliary tubercles.

RABBITS.

Inoculated on May 9, 1906 with culture from Dog 72.

Number.	Dose.	Method.	Duration of Life.	Result.
867	0·1 mg.	Intrav.	D. 26 days	G.T.
868	1·0 mg.	Intrap.	D. 19 „	G.T.
869	0·1 mg.	Intrap.	D. 8 „	Coccidiosis.
871	1·0 mg.	Subcut.	D. 109 „	G.T.
870	0·1 mg.	Subcut.	D. 55 „	G.T.

TABLE 15.

PIG PASSAGE.
VIRUS B I.
(May 6, 1902.)

PIG 8.

Fed with milk of Cow 40 for 92 days.
Killed, June 4, 1903, 247 days after the experiment began.
P.M.—Two or three caseous nodules in the small intestine, not ulcerated; enlarged and caseous mesenteric glands; slight tuberculosis of lungs, thoracic glands and liver.

Emulsion of mesenteric glands.

PIG 10.

Subcutaneous.

Dose: 10 cc. (no T.B. found).

Killed, October 8, 1903 (126 days).

P.M.—Small local lesion; adjoining glands caseous and calcareous; tuberculosis of all the organs except the spleen.

PIG 12.

Subcutaneous.

Dose: 10 cc. (no T.B. found).

Killed, October 3, 1903 (121 days).

P.M.—Caseous local lesion; neighbouring glands caseous and calcareous; general tuberculosis.

CALF 104.

Subcutaneous.

Dose: 10 cc. (no T.B. found).

Killed. September 11, 1903 (99 days).

P.M.—Calcareous tubercles at seat of inoculation; calcareous prescapular gland; tubercles in liver, spleen, lungs, thoracic and two mesenteric glands.

CALF 106.

Subcutaneous.

Dose: 10 cc. (no T.B. found).

Killed, September 12, 1903 (100 days).

P.M.—Calcareous tubercles at seat of inoculation; prescapular gland caseous and calcareous. One calcareous tubercle in a mediastinal gland.

Emulsion of inguinal and hepatic glands and spleen.

PIG 14.

Subcutaneous.

Dose: 9,000 T.B.

Killed, January 25, 1904, (114 days).

P.M.—Small ulcerated fibro-caseous lesion; enlargement and caseation of inguinal, iliac, and gastric glands; a few tubercles in liver, about 30 in each lung; spleen unaffected; bronchial glands tuberculous.

PIG 16.

Subcutaneous.

Dose: 9,000 T.B.

Killed, January 16, 1904, (105 days).

P.M.—Ulcerated local lesion; enlargement and caseation of inguinal, iliac, and gastric glands; numerous tubercles in liver. spleen, lungs, and bronchial glands; tubercles on the diaphragm, pleura and meso-testis.

CALF 124.

Subcutaneous.

Dose: 9,000 T.B.

Died, December 12, 1903 (71 days).

P.M.—General tuberculosis.

CALF 130.

Subcutaneous.

Dose: 9,000 T.B.

Killed, January 15, 1904. (104 days).

P.M.—Small calcareo-caseous tumour; adjacent glands tuberculous; slight tuberculosis of liver, spleen, lungs and thoracic glands.

CULTURE.

PIG 98.

Subcutaneous.

Dose: 1·0 mg.

Died, March 29, 1905, (78 days).

P.M.—Small local lesion; one inguinal gland partly caseous; a few tubercles in the liver and spleen, and numerous tubercles in the lungs which showed areas of consolidation; tuberculosis of pleura and diaphragm.

PIG 96.

Subcutaneous.

Dose: 0·1 mg.

Killed, September 7, 1905, 240 days after.

P.M. — Small caseous lesion; neighbouring glands caseous; spleen contained three miliary tubercles, the liver numerous caseous nodules and the lungs sparsely scattered hard tubercles; the thoracic and several of the abdominal glands, and two glands in the neck were tuberculous.

CALF 230.

Subcutaneous.

Dose: 50 mg.

Died, February 13, 34 days after inoculation

P.M.—General tuberculosis.

RABBITS.

No.	Dose.	Method.	Duration of Life.	Result.
159	1·0 mg.	Intrav.	D. 21 days.	G.T.
158	1·0 mg.	Intrap. partly subcut.	D. 62 „	G.T.

CULTURE.

RABBITS.

Number.	Dose.	Method.	Duration of Life.	Result.
558	1·0 mg.	Subcut.	D. 81 days	G.T.
559	0·1 mg.	„	D. 137 „	G.T.
560	0·01 mg.	„	D. 106 „	G.T.
561	0·001 mg.	„	D. 50 „	G.T.

DETAILED DESCRIPTION OF EXPERIMENTS.

CHIMPANZEE PASSAGE.

VIRUS B. IV.

CHIMPANZEE 8.*

Fed on October 25th, 1904, with 107 c.c. milk of Cow 172, containing approximately 10,000,000 tubercle bacilli.

Died of broncho-pneumonia, March 21st, 1905. 144 days later.

Abstract of Post-Mortem Notes.

Pharynx and Glands in Neck normal; the small intestine showed five tuberculous ulcers, varying from 3 to 4 mm. up to 2 cm. in diameter. Nine of the mesenteric glands were slightly enlarged and contained dense fibroid nodules, caseous in the centre and appreciably gritty from calcification. In the meso-colon there were three enlarged glands, close together; two were caseous throughout and slightly gritty, the third was partly caseous. There was an abscess (not tuberculous) in the wall of the abdomen and broncho-pneumonic patches in the lungs.

A culture was isolated from one of the colic glands and inoculated into a series of six rabbits and chimpanzee 10 (by feeding).

Two of the rabbits died prematurely; the rest died of general tuberculosis in periods varying from 16 to 64 days.

CHIMPANZEE 10.

(SECOND CHIMPANZEE PASSAGE OF VIRUS B. IV.)

Fed with culture derived from a colic gland of Chimpanzee 8.

Dose.—0·1 milligramme (4th generation, 24 days old).

Date.—July 16th, 1905.

Weight.—July 18th, 1905. 11 lbs. 14 ozs.

Died.—November 6th, 1905. 113 days after the experiment began.

Clinical History.

Temperature.—The temperature during the first six weeks varied between 37° and 38·4°, with the exception of two rises each to 39·1° on the twentieth and fortieth days respectively. On August 27th (42 days after feeding) there was a sudden rise of temperature to 40·3° C., and a rapid fall on the next day to 36·5° C. The temperature rapidly rose again, and varied for about a fortnight between 37·9° and 39·3°; it then gradually became more and more irregular, and in the month of October showed considerable fluctuations, on several occasions rising more than 2°, and on two falling more than 3° C. (highest temperature 39°C.). In the last few days of life no temperature was recorded.

With the rise of temperature on August 27th the Chimpanzee was a little seedy for a day or two (loss of appetite, and depression); he recovered, but remained somewhat anæmic. During the month of October he gradually lost flesh and strength, and became extremely anæmic.

In the last week of life weakness and emaciation were extreme, and the eyelids in the morning were very œdematous.

No diarrhœa was noticed during the experiment, and there was no increase in respiration. On one occasion only was he heard to cough.

Weights.—During the first week there was a gain of 10 ozs.; then for a fortnight the weight was stationary; subsequently the animal steadily lost weight, at first slowly, then rapidly.

July 18th, 1905	- - - 11 lbs.	14 ozs.
July 25th, 1905	- - - 12 lbs.	8 ozs.
August 1st, 1905	- - - 12 lbs.	8 ozs.
August 8th, 1905	- - - 12 lbs.	8 ozs.
September 12th, 1905	- - 12 lbs.	2 ozs.
October 5th, 1905	- - - 12 lbs.	0 ozs.
October 10th, 1905	- - - 11 lbs.	14 ozs.
October 24th, 1905	- - - 11 lbs.	0 ozs.
November 6th, 1905	- - 9 lbs.	12 ozs.
Loss in weight on the original weight	- - - - 2 lbs.	2 ozs.
Average loss per week from August 8th to November 6th	- 3·43 lbs.	

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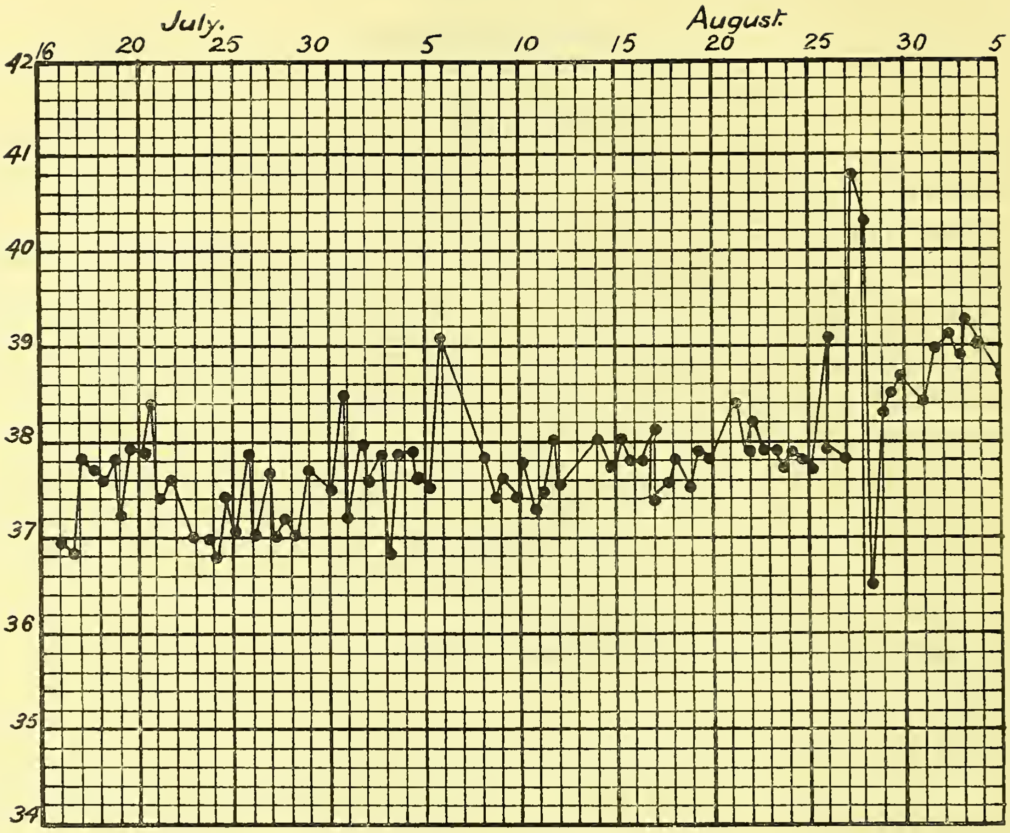
* In the majority of these experiments the first animal of the series belongs to one or other of the feeding experiments and a full description of the post-mortem results will be found in Volume I.; for convenience of reference a short abstract of the notes of these animals is given at the beginning of each series.


July.
August.
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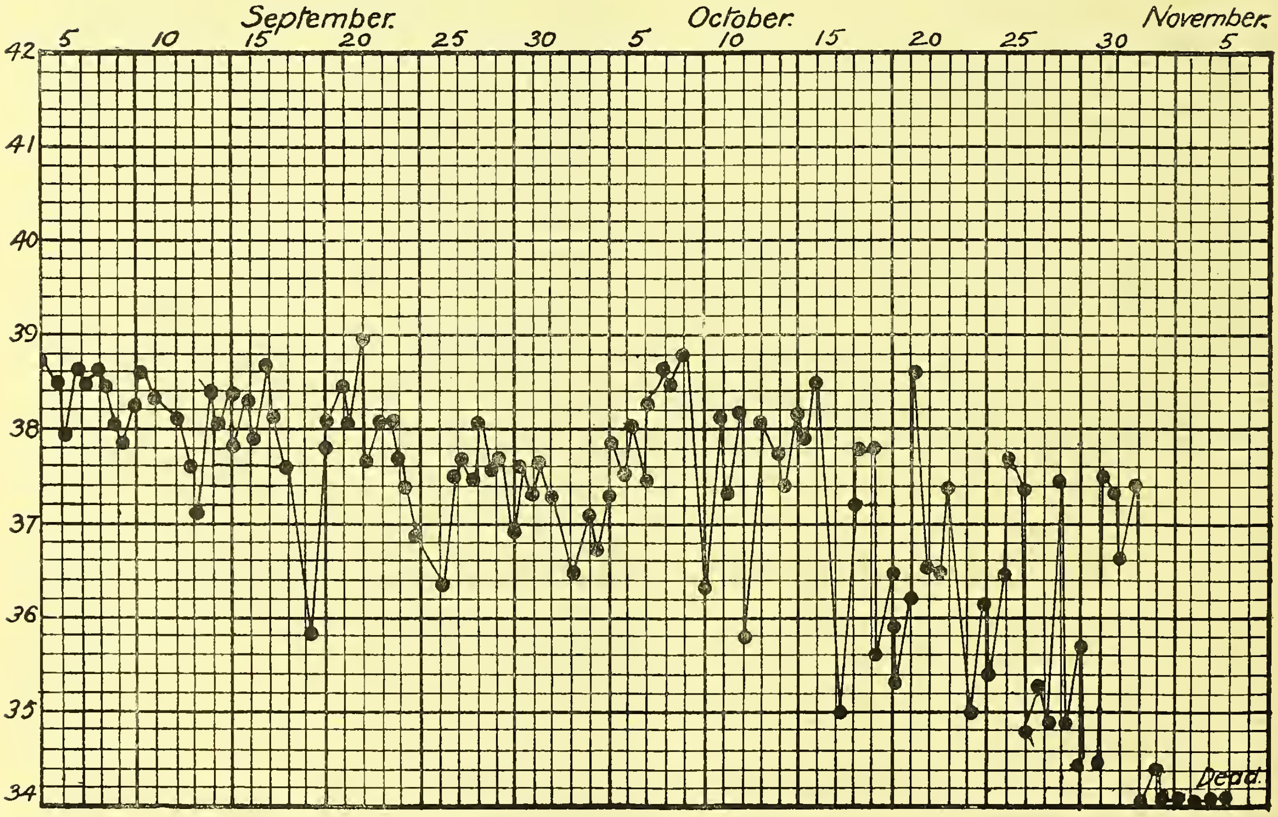
September.
October.
November.
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42
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35
34
Dead.

Post-Mortem Examination.

Body.—Emaciated.

Tongue, Tonsils, Pharynx, Submental Glands, Submaxillary Glands, Parotid Glands, Retro-pharyngeal Glands.—Normal.

Cervical Glands.—Left upper cervical: one enlarged gland showed irregular caseous areas replacing most of the cortex; two smaller glands contained yellowish white softened nodules, each the size of a wheat grain.

Left lower cervical glands normal.

Right upper cervical; one showed in the cortex two softened caseous nodules, each 2 mm. in diameter; the others were normal.

Right lower cervical glands normal.

Abdomen.

Peritoneum.—There was a slight excess of fluid in the peritoneal cavity

Omentum.—The omentum along its attachment to the stomach was œdematous: it contained minute scattered pearly grey tubercles.

Stomach.—Normal.

Small Intestine.—The wall of the intestine was reduced in thickness, and in the situations of some of the larger ulcers presented saccular dilatations. The mucous membrane showed about fifty ulcers distributed along the whole length of the gut, the first situated five inches from the pylorus; twenty-five of these were large, ranging from 1 by 1 cm. to about 2·5 by 2 cm. in area (the latter almost completely encircled the gut); the rest were quite small.

The margins of the ulcers were raised, and the bases slightly thickened and fibrous, showed on the serous surface in many cases an eruption of hard raised tubercles, some pearly grey, others slightly opaque in the centre. In the larger ulcers the floors were irregular and somewhat nodular. None of the ulcers showed caseous deposit, but in some a few minute opaque foci were seen in the base and under the surrounding mucous membrane.

Around many of the larger ulcers there were numerous small ulcers; these in some cases had united with the large ulcer, causing the latter to have a sinuous outline.

Some of the Peyer's patches in the ileum were not uniformly ulcerated, but showed a number of small irregular ulcers, the mucous membrane between which was raised and thickened.

Large Intestine.—Showed one small ulcer, with slightly thickened margin and thin base; no subperitoneal tubercles.

Gastric Glands.—A small gland near the pylorus contained a soft white caseous nodule 1·5 mm. in diameter; the rest were normal.

Mesenteric Glands.—The mesenteric glands were all much enlarged; those in the upper part of the mesentery were fused together, and formed a firm nodular mass $1\frac{3}{4}$ in. by $1\frac{1}{4}$ in. by (about) 1 in.; the glands in the lower part of the mesentery were more or less moveable. On section through the large mass all the glands were seen to be caseous throughout and partially softened. They had thick fibrous capsules. which adhered together. The mesentery between this mass and the intestine was œdematous. The glands in the lower part of the mesentery were similarly affected; the caseated tissue was opaque, whitish, firm in most cases, but in some beginning to soften.

Around the margins of the mass of caseated glands were several œdematous glands, one of which contained a caseous focus.

Ileo-Colic Glands.—At the junction of the ileum and the colon there was a group of enlarged glands, some of which were normal on section, whilst others showed the substance replaced to a greater or lesser extent by opaque white caseated tissue, in one or two cases beginning to break down; one gland, the size of a large pea, was caseous throughout.

Colic Glands.—One colic gland contained a soft white caseous tubercle the size of a millet seed; another, the size of a small pea, was caseous nearly throughout. Other colic glands along the margin of the bowel appeared somewhat enlarged, but were not otherwise abnormal.

Spleen.—Was much enlarged ($4\frac{1}{2}$ in long by $1\frac{3}{4}$ in. broad), and was adherent to the wall of the abdomen at the level of the costal cartilages by a tough fibrous adhesion. On the surface were fairly numerous filmy grey tubercles, the largest a little over 1·5 mm. in diameter. On section the pulp contained moderately numerous spherical tubercles, each about one millimetre in diameter, with yellow softened centres and grey translucent margins. There were besides two or three soft caseous nodules with fibrous margins, the largest the size of a wheat grain.

Liver.—The substance was firm but very pale. On the surface were a few minute pearly grey foci, one of which was definitely raised above the surface. On section a few doubtful yellow points were seen.

Hepatic Lymphatic Glands.—A small gland on the duct from the gall bladder contained an opaque white pin-head sized tubercle. Two small glands on the common bile duct were normal.

Kidneys.—Were extremely pale; the capsules stripped readily. The left kidney showed on the surface about half a dozen slightly raised almost transparent tubercles, the largest about one millimetre in diameter. The right kidney showed a few similar tubercles.

Suprarenal Bodies.—Normal.

Pancreatic Glands.—On the upper border of the pancreas, near the duodenum, there was a small gland with a pin-head sized caseous tubercle. Three others further along the pancreas, each the size of a pea, were almost entirely composed of dense opaque white, or yellowish-white, caseated tissue.

Below the head of the pancreas there was a number of glands, ranging in size from a small pea to that of a swallow's egg; some of these were practically caseous throughout, others were partly caseous.

Lumbar Glands.—One on the right side, the size of a large pea, was indurated and extensively caseated; two or three other small glands contained each a caseous tubercle.

Thorax.

Pleura.—The parietal pleura was normal; the lymphatic fringes around the tendon of the diaphragm were slightly hypertrophied.

Lungs.—Were mottled with irregular purplish patches of congestion (pieces floated in water), and were closely and evenly beset with firm nodules, ranging in size from a pin's head to a small pea; the nodules were grey and translucent, the small ones showing a minute central opacity, the larger ones scattered opaque yellowish white points; some of the larger nodules were homogeneous throughout.

Bronchial Glands.—Were perhaps a little enlarged, but there were no foci of caseation.

The tissues of the posterior mediastinum were œdematous.

Heart.—The pericardial sac contained a quantity of clear serous fluid; the heart muscle and valves were normal.

Testicles.—Normal.

Iliac Glands, Inguinal Glands, Axillary Glands.—Normal.

Brain.—Normal.

Microscopical Examination.

Spleen.—Two tubercles were examined; no tubercle bacilli were seen, but there were a few organisms stained blue.

Left Superior Cervical Gland.—Two tubercle bacilli.

Pancreatic Gland.—Numerous tubercle bacilli.

Animals Inoculated.

With Tissue Emulsion.

Two guinea pigs, No. 1768 and 1769, were inoculated with an emulsion made from the lung; two, Nos. 1770 and 1771, from a mesenteric gland, and one, No. 1772, from the spleen; all died of general tuberculosis in periods varying from 15 to 40 days.

With Culture.

Seven rabbits inoculated with culture derived from a mesenteric gland died of general tuberculosis.

CHIMPANZEE 16.

Fed with culture from Chimpanzee 10.

Dose—0·01 milligramme.

Date—January 5th, 1906.

Weight—24 lbs. 10 ozs.

Died—April 6th, 1906. 91 days after the experiment began.

Clinical History.

The chimpanzee was seedy for the first three or four days, with diarrhœa and loss of appetite; the indisposition appeared after, and was probably due to the inoculation of tuberculin (2 cc.) on the day previous to the experiment.

The chimpanzee then remained in good health until about January 27, 1906, when she caught cold, probably from the attendant who was suffering from nasal catarrh at that time. During the next few days the chimpanzee lost appetite and ate nothing but fruit, and there was a constant discharge of muco-pus from the nostrils. Towards the end of this illness a cough developed and numerous râles and rhonchi were heard in the chest.

On February 5, 1906, she began to improve, and the appetite returned, but for a day or two there was still a hoarse cough.

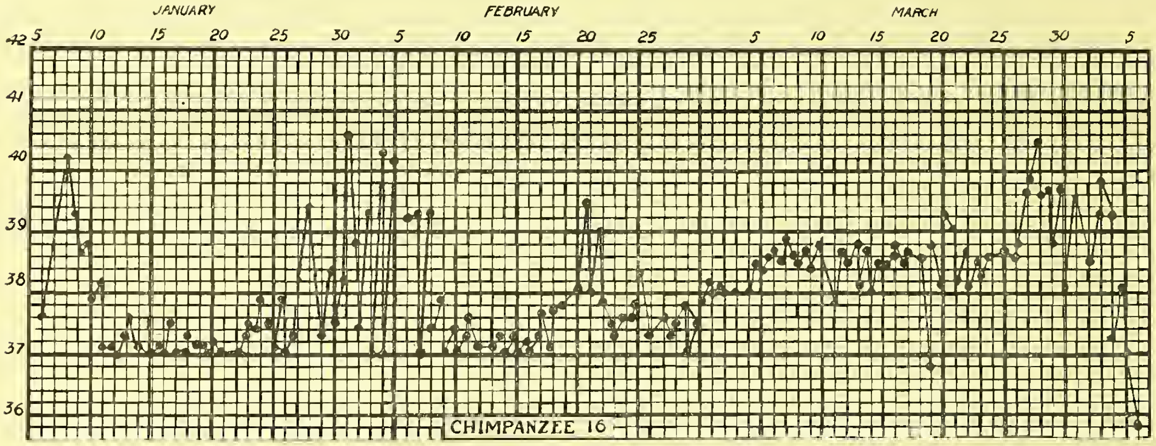
She remained well until March 12, 1906, when for a week she took scarcely any food in the morning and was much quieter than usual. She soon became obviously ill and gradually lost flesh and strength; she showed an increasing disinclination to exert herself and lay most of the day on her back.

On April 5, 1906, she was very weak and languid; respirations were not increased, but were accompanied in expiration by wheezing, probably from hypostatic congestion. She died on April 6, 1906, 91 days after she was fed.

Weights.

January 8th, 1906	24 lbs.	0 ozs.
January 25th, 1906	25 lbs.	2 ozs.
February 6th, 1906	24 lbs.	2 ozs.
February 21st, 1906	25 lbs.	8 ozs.
March 20th, 1906	24 lbs.	4 ozs.
April 6th, 1906	19 lbs.	4 ozs.
Loss in weight	4 lbs.	12 ozs.

Temperature.



POST-MORTEM EXAMINATION.

Body.—In fair condition but thinner than at beginning of experiment; moderate amount of fat in subcutaneous tissues.

Tongue, Tonsils and Pharynx.—Normal.

Submental, Submaxillary and Parotid Lymphatic Glands.—Normal.

The Right Pharyngeal Gland.—The size of a French bean, showed about three-quarters of its substance yellow, caseous and softened.

The Left Pharyngeal Gland was normal.

Cervical Glands.—On the right side one in the middle of the neck contained two yellow caseous miliary tubercles; the rest were normal. On the left side two small glands in the middle of the neck contained each a soft caseous nodule 1 to 1·5 mm. in diameter; other glands on this side were normal.

Abdomen.

There was a quantity of blood-stained serous fluid in the peritoneal cavity.

The Omentum.—Was moderately fatty and contained fairly numerous grey tubercles, somewhat irregular in outline, the largest a little over 1 mm. in diameter; most of these were translucent throughout, but a few were slightly opaque in the centre; there were besides a few larger ones with soft caseous centres.

Peritoneum.—The peritoneum covering the diaphragm and the lower ribs on the left side was covered with flattened grey tuberculous growths, forming on the tendinous part of the diaphragm an almost continuous layer and showing here and there opaque foci; elsewhere the parietal peritoneum was somewhat roughened, mainly on the left side, and showed one or two small tubercles with caseous centres.

On the meso-colon there were sparsely scattered miliary caseous tubercles.

The appendices epiploacæ were devoid of fat and along the first parts of the colon contained a varying number of hard grey tubercles, most of which were caseous in the centre.

Stomach.—Mucous membrane covered with mucus and partly digested; otherwise normal.

Small Intestine.—The duodenum below the pancreas was closely adherent to the enlarged mesenteric glands and along its mesenteric attachment was thickened and caseating; all the coats were infiltrated except the mucous which was not ulcerated.

The mucous membrane of the small intestine in the upper part was normal except for a flattened caseous nodule, about 5 mm. in diameter, with a small central ulcer.

About three feet from the pylorus there were two tuberculous ulcers close together, one nearly 1 cm. in diameter, the other smaller and more transverse; under the mucous membrane around the ulcers there were a few small caseous nodules, extending chiefly in a lateral direction. On the serous surface opposite these ulcers and for a good distance around them there was an eruption of caseating miliary tubercles.

Most of the Peyer's patches, from about the middle of the small intestine to its termination, were affected; five contained each a typical tuberculous ulcer with raised irregular caseous margins and slightly thickened fibroid base; on the serous surface opposite there were two or three hard tubercles; the ulcers varied from 4 to about 15 mm. in greatest diameter, the one in the terminal patch being larger and more irregular than the others; in the latter patch besides the ulcer there was a slightly raised yellow caseous nodule, the size of a split pea, with a central ulcer. The other affected Peyer's patches contained from one to six yellow caseous nodules, ranging in size from 3 to 6 mm. in diameter; these resembled the ulcerated nodule in the terminal patch. Another patch contained one minute tubercle.

Large Intestine.—The mucous membrane showed numerous small hæmorrhages but no ulcers or nodules.

Gastric Glands.—Præcardiac glands normal. A gland in the lesser curvature of the stomach contained one soft yellow caseous nodule, 5 mm. in diameter and two small caseous tubercles; a smaller gland contained a miliary caseous tubercle.

Mesenteric Glands.—The mesenteric glands were much enlarged. In the upper part of the mesentery they were fused together to form a tumour, measuring 3 by 2 by $3\frac{3}{4}$ in. closely adherent at one part to the duodenum; the mass was nodular around the margins and showed on the peritoneal surface numerous slighty raised grey tubercles, the larger of which were slightly opaque in the centre; on section the glands were caseous and softened throughout and had thickened capsules. In the lower part of the mesentery there was a smaller mass, measuring $1\frac{1}{2}$ by $\frac{3}{4}$ by $\frac{3}{4}$ in., the glands composing which were more or less moveable one over the other; on section they consisted of softened caseous material with a slightly greenish tinge. Between this mass and the ileo-colic glands there were two slightly enlarged glands each of which contained an irregular softened caseous nodule, the largest 4 mm. in diameter.

Ileo-colic Glands.—On one side of the junction of the ileum and cœcum there was a mass of glands measuring $1\frac{1}{2}$ by 1 by $\frac{3}{4}$ in.; all these were caseous and softened throughout with the exception of a few small œdematous glands at one extremity which were not obviously tuberculous.

On the opposite side of the cœcum at the root of the appendix there was a group of three small glands; two were entirely caseous, the third contained discrete caseous tubercles.

Colic Glands.—Three colic glands, two on the ascending and one on the descending colon, were caseous and softened throughout; they ranged in size from a small to a large pea; all the other numerous small glands along the large intestine were normal.

The Cœliac and Lumbar Glands.—Were much enlarged, and formed an irregular mass situated between the pancreas and the vertebral column; the lumbar glands were all caseous throughout, whilst some of the cœliac glands were only partly caseous.

In close contact with the upper border of the head of the pancreas, there were two enlarged glands; the larger one contained several soft yellow caseous nodules, and some small tubercles; the smaller one showed an irregular caseous patch replacing about a quarter of the substance.

Spleen.—The spleen was much enlarged, measuring $5\frac{1}{2}$ in. by $2\frac{1}{2}$ in. by about 1 in. in greatest thickness. Projecting from the surface were numerous yellow nodules ranging in size from 1 to 5 or more millimetres in diameter; most of these were surrounded by broad, deeply congested, fibrinous fringes. On section the pulp was firm and dark red, and showed numerous soft yellow caseous nodules, up to 2 or 3 mm. in greatest diameter; these nodules were very irregular in outline, and in several places had coalesced or formed small groups; besides the yellow nodules there were numerous greyish tubercles, some just visible to the eye.

Splenic Lymphatic Glands.—One gland in the hilum of the spleen contained two or three irregular caseous tubercles; another showed a few caseous foci.

The Liver.—Was enlarged, soft and of a pale yellowish colour; on the surface there were numerous slightly raised, flattened tubercles, ranging from about 1 to 2 mm. in diameter; the smaller tubercles were transparent and glassy; the medium sized ones had minute opaque centres, whilst the larger ones were distinctly yellow in the centre. On section small caseous tubercles, the largest about 1 mm. in diameter were sparsely scattered throughout the substance; the cut surfaces showed also numerous transparent grey points.

Portal Glands.—In the portal fissure there were two glands, ranging in size from a pea to a kidney bean; one was caseous throughout; the other contained soft, caseous nodules.

The Kidneys.—Were very pale and appeared to be enlarged; each showed on the surface scattered punctiform hæmorrhages, and four or five miliary tubercles with irregular caseous centres and ill-defined grey margins; no tubercles were seen on section.

Suprarenal Bodies.—Normal.

Thorax.

Heart.—The pericardial sac contained a small quantity of blood-stained fluid; the pericardium was normal. The heart muscle under the pericardium was deeply hæmorrhagic; endocardium normal.

Pleura.—On the pleural surface of the diaphragm, on the left side, there were four flattened caseating nodules, the largest 3 mm. in diameter; the pleura on the muscular part of the diaphragm was covered with minute grey granules. Costal pleura normal.

Lungs.—The left upper lobe was slightly adherent to the chest wall; the lobes of each lung were adherent to each other by old fibrous adhesions. The ventral part of the left upper lobe was firm, dark red, and airless, and there were some red hepatised patches around the roots of the right middle and the left lower lobes; with these exceptions the lungs were crepitant. The lung tissue contained sparsely scattered hard tubercles, ranging in size from about 0·5 to 3 mm. in diameter; the tubercles showed a small amount of central caseation.

Bronchial Glands.—On the right side, one above the bronchus contained an irregular caseous tubercle, 1·5 mm. in diameter; others on this side were normal. On the left side one small gland above the bronchus showed a minute caseous tubercle; a gland below the bronchus on this side contained two miliary caseous tubercles; the rest appeared normal.

Under the pleura in the last interspace near the vertebræ, there was a soft caseating mass, the size of a broad bean.

Brain and Meninges.—Normal.

Various Lymphatic Glands.

One Right Iliac Gland was slightly enlarged, and partly caseous; other iliac glands were normal.

Inguinal Glands.—On the right side one contained two soft, yellow caseous nodules, 3 and 5 mm. in diameter; another contained a minute caseous tubercle; on the left side one contained a soft caseous nodule, 2 mm. in diameter; the rest were normal.

Axillary Glands.—Three glands on the right side contained each a miliary caseous tubercle; another contained a small soft caseous nodule. A gland on the left side was slightly enlarged and showed more than half its substance caseous and softened; other glands in this group appeared normal.

Microscopical Examination (Smear Preparations).

Nodule in Omentum.—Tubercle bacilli moderately numerous.

Inguinal Gland.—Tubercle bacilli moderately numerous.

Left Cervical Gland.—Tubercle bacilli moderately numerous.

Portal Gland (Emulsion).—Tubercle bacilli moderately numerous.

Spleen (Emulsion).—A few tubercle bacilli.

Animals Inoculated.

With Tissue Emulsion.

Two guinea-pigs inoculated subcutaneously, one from the spleen, the other from a lumbar gland, died of general tuberculosis in 32 and 31 days respectively.

Two rabbits inoculated subcutaneously, one with an emulsion from the spleen, the other with an emulsion from a lumbar gland, died of general tuberculosis in 67 days.

With Culture.

A culture isolated from the spleen of Chimpanzee 16 was used to inoculate a series of rabbits and Chimpanzee 22.

One of the rabbits died prematurely; all the others developed tuberculosis.

CHIMPANZEE 22.

Subcutaneous Inoculation of Culture (5th generation, 21 days old) derived from Chimpanzee 16.

Date—July 4th, 1906.

Dose—0·001 milligramme.

Died—September 29th, 1906.

Duration of Experiment—87 days.

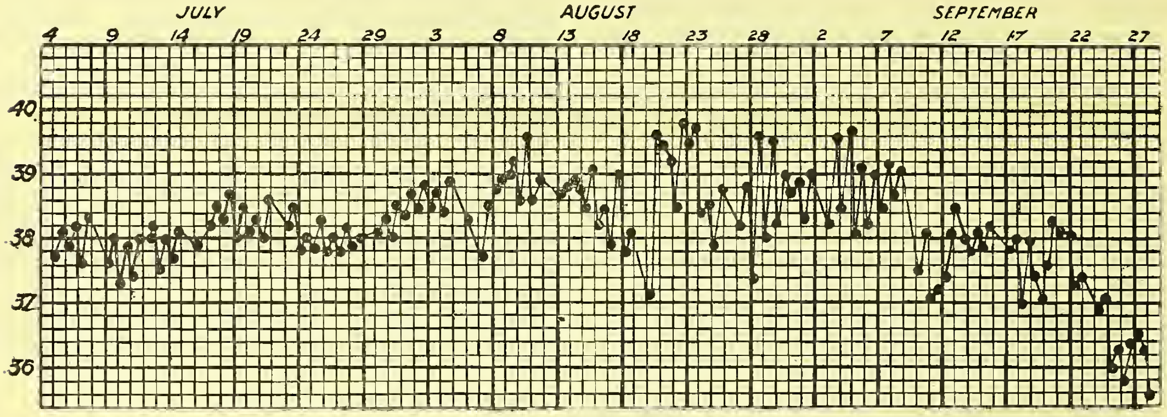
Clinical History.

The chimpanzee was inoculated in the subcutaneous tissue of the back on July 4th, 1906. One month later there was a soft slightly raised tumour about 2 in. in diameter, with a small ulcer in the skin at one margin from an opening in the base of which issued a purulent discharge: the axillary glands were not enlarged. Subsequently the ulcer in the skin became larger and the axillary glands enlarged. The first sign of ill-health appeared six weeks after inoculation and was shown by loss of appetite. From that time it slowly and progressively deteriorated in condition until its death.

Weights.

July 5th, 1906 - - -	13 lbs. 9 ozs.
September 29th, 1906 - -	9 lbs. 8 ozs.
Loss in weight - - -	4 lbs. 1 oz.
Average weekly loss - -	5·41 ozs.

Temperature.



POST-MORTEM EXAMINATION.

Condition.—Body thin; muscles of extremities wasted; very little subcutaneous and abdominal fat.

Local Lesion.—Just to the right of the middle line of the back and lying to some extent on the ala of the right scapula there was a slightly raised tumour measuring 1½ by 1¼ by ½ in.; on section the tumour was found to be composed of muscular tissue closely beset with irregular caseous patches and tubercles and to contain near the skin a small compressed cavity lined internally with a thin layer of caseous substance; this cavity had leading from it to the surface a short sinus which opened at the margin of an ulcerated area ¾ in. in greatest diameter. The deeper muscles under the tumour were found to contain caseous tubercles and a soft caseous cord could be traced through an intercostal space into the thorax.

Axillary Glands.—In the right axillary region at about the middle of the margin of the pectoralis major there was a tumour measuring 1½ by 1 by ½ in. composed of several glands which had fused together; on section these glands consisted throughout of yellowish-white breaking down caseous substance; around this mass were numerous small glands, one of which was beset with minute caseous foci. Near the large mass, but lying more under the muscle, were two enlarged glands, each about 1 cm. in diameter, which showed on section yellowish white caseous patches of various sizes, undergoing softening. Near the apex of the axilla were four or five slightly enlarged glands; the largest of these was partly caseous, the others contained discrete caseous foci. The glands in the left axilla were normal with one exception, which contained a soft caseous nodule the size of a hempseed.

Thorax.

On opening the thorax the left lung collapsed and was nowhere adherent to the chest wall; the right lung was universally adherent to the parietal pleura, but could be fairly readily separated from it. The parietal pleura (costal and diaphragmatic) on the right side was thickened, greyish-yellow in colour and roughened on its internal surface.

Lungs.—Both lungs were crepitant; the lobes of the right lung were adherent.

The left lung contained moderately numerous evenly distributed hard grey nodules ranging in size from a pin's head to that of a hempseed; most of the smaller nodules were quite translucent, whilst the larger ones showed a relatively small amount of central caseation; the right lung contained similar but rather less numerous nodules.

Bronchial Glands.—On the right side the prætracheo-bronchial glands (three in number) were fused together to form a mass the size of a thrush's egg; on section they were yellow and caseous throughout and had thick fibrous capsules. On the left side one gland, the size of a sparrow's egg, showed about three-quarters of its substance replaced by soft yellow caseous substance.

The intertracheo-bronchial glands were normal.

The vertebral glands on the right side extending from the fourth to the last dorsal vertebra were enlarged, caseous and softened and embedded in dense white fibrous tissue.

A dorsal mediastinal gland at the level of the fifth vertebra was large, caseous and softened throughout.

A lymphatic gland on the diaphragm near the reflexion of caval fold of pleura contained a soft caseous tubercle.

Diaphragm.—The pleura on the left side of the diaphragm and the peritoneum on the right showed a number of filmy translucent growths varying in size up to about 3 mm.

Heart.—Normal.

Abdomen.

The omentum was devoid of fat and somewhat œdematous; it contained fairly numerous tubercles varying in size from a mere speck up to that of a millet seed.

Spleen.—The spleen was enlarged and measured 4 in. by 2¼ in. by about ½ in. in greatest thickness; on section the pulp contained numerous irregular soft yellow caseous areas ranging from about 2 to 10 mm. in diameter; the larger nodules were composed of firm reddish tissue (firmer than the surrounding splenic pulp) containing soft yellow caseous areas; two of the smaller nodules projected from the surface and were surrounded by a fringe of congested fibrin.

Splenic Lymphatic Glands.—In the hilum of the spleen there were two enlarged glands, one the size of a broad bean, the other the size of a kidney bean; both on section showed irregular softened caseous areas extensively replacing the gland substance.

Liver.—The liver was paler than normal and showed on the surface about twenty flattened nodules, with overhanging margins, the largest 5 mm. in diameter; on section they were found to extend into the substance and to have yellow caseous centres and grey margins.

Under the capsule and throughout the substance were seen scattered tubercles up to 1 mm. in diameter; some of these were grey with opaque centres, whilst others were homogeneously caseous.

In the small omentum there was a group of four enlarged glands, the largest the size of a broad bean, which on section showed whitish caseous patches extensively replacing the gland substance.

In the hilum of the liver there was a gland, 1 cm. in diameter, similarly affected.

A gland near the neck of the gall-bladder contained a softened miliary caseous tubercle.

Kidneys.—The kidneys were extremely pale. The right showed in the cortex a pinhead-sized caseous focus. In the cortex of the left there were five or six nodules, the largest about 4 mm. in diameter; one of these appeared to be composed of three smaller nodules which had fused together and was completely caseous; the others had grey margins and irregular caseous centres.

Tongue, Pharynx, Tonsils, Larynx and Trachea.—Normal.

Small Intestines.—Three Peyer's patches in the ileum contained each a caseous focus.

Large Intestine.—Normal.

Gastric Glands.—A gland in the lesser curvature of the stomach was beset with soft caseous tubercles.

Mesenteric Glands.—In the anterior part of the mesentery a gland the size of a large pea was irregularly caseated; two smaller glands near the latter were extensively replaced by soft caseous nodules; two other glands in the middle of the mesentery contained each a miliary caseous tubercle.

Ileo-colic Glands.—Normal.

Colic Glands.—Two colic glands were affected; one contained two miliary caseous tubercles, the other contained one.

Lumbar Glands.—One lumbar gland contained a minute caseous focus; the others were normal.

Inguinal and Iliac Glands.—Normal.

Testicles and Brain.—Normal.

Microscopical Examination (Smear Preparations).

Spleen.
Bronchial Gland, } Numerous tubercle bacilli.

Intestines.—A few tubercle bacilli.

CHIMPANZEE PASSAGE.

Virus B. IX.

CHIMPANZEE 14.

Fed on September 11th, 1905, with 1·1 mg. of culture derived from the original material of Virus B. IX.

Died—November 13th, 1905. 63 days after.

Post-mortem Examination.

General tuberculosis; intestines and mesenteric glands severely affected.

A culture isolated from an ileo-colic gland was used to feed Chimpanzee 18 and to inoculate subcutaneously a series of rabbits.

One of the rabbits died in 25 days and showed slight tuberculosis; the others died of general tuberculosis in periods varying from 103 to 151 days.

CHIMPANZEE 18.

Fed with culture derived from Chimpanzee **14.**

Date—February 12th, 1906.

Dose—0·01 milligramme.

Weight—11 lbs. 8 ozs.

Died—June 8th, 1906. 116 days later.

Clinical History.

Towards the end of March a mass of enlarged glands was felt on the left side of the neck.

On April 19, 1906, the chimpanzee had a sharp attack of diarrhœa.

Note on April 29, 1906: "For three days has been very weak and apparently unable to walk about the cage; the weakness is manifest chiefly in the legs. The appetite is poor and has not been good for several days. The mass of glands completely fills the anterior triangle and enlarged glands are felt in the posterior triangle as well. The axillary glands are not perceptibly enlarged and no enlarged glands can be felt in the abdomen."

On May 4, 1906, a view was obtained of the left tonsil which was observed to be congested and ulcerated. The skin over the lower part of the mass of enlarged glands was slightly reddened but not adherent. The general condition of the animal was moderate and the appetite was fairly good; the chimpanzee was, however, much quieter and moved very little about the cage. During the rest of the experiment the chimpanzee gradually became more depressed and more and more disinclined to move about; the appetite was capricious but usually a fair quantity of food was taken and there was no apparent bodily wasting. On several mornings there was oedema of the eyelids and of the skin over the lower part of the glandular mass. There was no increase in the rate of respiration during the experiment.

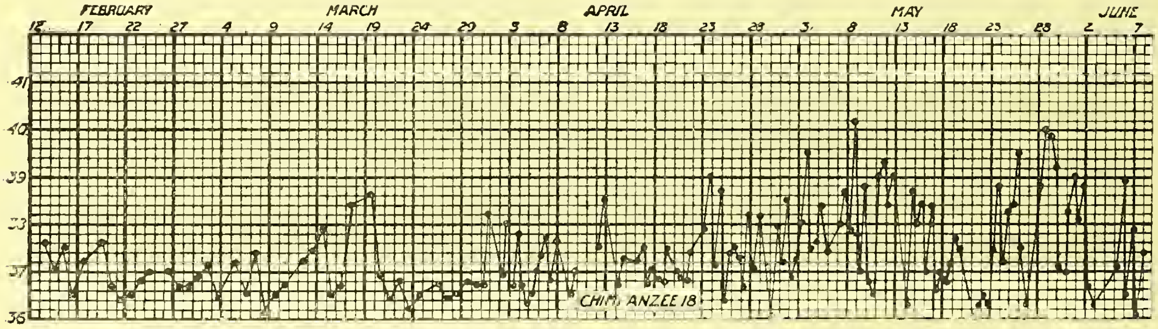
The chimpanzee died rather unexpectedly on June 8, 1906, 116 days after the experiment began.

Weights.

February 21st, 1906 - - -	11 lbs. 8 ozs.
March 20th, 1906 - - -	12 lbs. 6 ozs.
April 18th, 1906 - - -	11 lbs. 2 ozs.
May 15th, 1906 - - - -	11 lbs. 0 oz.
June 5, 1906 - - - -	9 lbs. 8 ozs.
June 8, 1906 - - -	10 lbs. 4 ozs.
Loss in weight - - -	1 lb. 4 ozs.

Tuberculin Test.—April 10th, 1906. Fifty-seven days after the experiment began. Positive. Rise of temperature 2·3° C. Maximum 40·5° C.

Temperature.



Post-mortem Examination.

Condition.—Arms and legs thin; muscles poorly developed; abdomen full, not distended.

Tonsils.—In the region of the left tonsil there was a deep ulcer with caseo-necrotic walls. The right tonsil was not apparently ulcerated. the surface was irregular and the crypts were filled with whitish purulent material.

Pharynx and tongue appeared normal.

Lymphatic Glands of the Neck.—In the left anterior triangle there was a large mass of enlarged glands extending from the level of the angle of the jaw to the clavicle; in the upper part of the triangle the glands, superior cervical and retropharyngeal, had fused together and formed a soft fluctuating tumour the size of a small orange; over this swelling the sterno-mastoid muscle was stretched; on section the tumour was filled with breaking down caseous material: the glands between this tumour and the clavicle (lower cervical) were enlarged and caseous; around the fluctuating tumour were several isolated enlarged glands; some of these were caseous throughout; others showed caseous patches.

In the left posterior triangle there was a smaller mass of fused glands; on section all were caseous throughout and some were softened.

On the right side in the anterior Triangle there was a mass of glands (superior cervical) measuring $1\frac{1}{4}$ by $\frac{3}{4}$ by $\frac{1}{2}$ in.; on section the glands composing the mass were caseous throughout and beginning to soften. Below this there was a chain of slightly enlarged glands running under the sterno-mastoid; on section these were free from caseation. The retropharyngeal gland was not apparently affected.

Abdomen.

On cutting through the wall of the abdomen the omentum was found to be very intimately adherent to the parietal peritoneum, in several places so firmly that it was impossible to separate them one from the other.

The Peritoneal Cavity.—Contained a small quantity of slightly turbid fluid.

The Omentum.—Was studded with greyish-white somewhat translucent nodules, ranging in size from a mere point to that of a hempseed; some of the larger of these showed minute opaque points in the centre, the others were homogeneous throughout. Along the free margin of the omentum the nodules were aggregated together and formed irregular nodular masses composed of a firm greyish translucent tissue containing sparsely scattered caseous foci.

The Mesentery.—Was covered with similar nodules, the largest the size of a hempseed, here and there aggregated together; a few of the larger ones were definitely caseous in the centre.

There were a few similar tubercles on the serous surfaces of the stomach and small intestine.

On the large intestine there were rather numerous nodules along the line of attachment of the mesocolon; there were numerous nodules also on the mesocolon itself; near the junction of the ileum and cœcum the nodules were closely aggregated together.

All the appendices epiploacæ on the large intestine were devoid of fat and filled with greyish-white miliary tubercles, which in several had fused together to form firm nodular masses of various sizes.

The Parietal Peritoneum.—Was covered with flattened nodules similar in appearance to those on the mesentery; on the anterior abdominal wall they formed an almost continuous greyish-white layer with a yellowish mottling; in the flanks they had united together to form irregular nodular masses similar to those in the omentum; to these masses the large intestine was adherent.

The Peritoneal Surface of the diaphragm showed on the right side irregular plaques of aggregated tubercles, and on the left a uniform greyish-yellow layer of tuberculous tissue; the latter extended over the peritoneum covering the ribs.

Stomach.—Normal.

Small Intestine.—The upper part of the small intestine was normal. Twelve of the Peyer's patches in the ileum showed typical tuberculous ulceration; the ulcers varied from about 5 to 10 mm. in diameter and were mostly circular; the ulcers in the first six patches were caseous chiefly under the margins, two only showing caseous tubercles in their thickened fibroid base; in one of the patches, besides a large ulcer, there were three caseous nodules, the largest of which was ulcerated; on the peritoneal surface of one of the ulcers there were a few raised tubercles; five of the patches in the lower part of the ileum were enlarged, yellow and caseous throughout, and presented nodular elevations on the serous surface; the mucous membrane over some of the patches was almost completely ulcerated, in others only slightly.

The terminal Peyer's patch showed one small ulcer with caseous margins. The large intestine was adherent at several places to the wall of the abdomen; the mucous surface appeared normal.

Gastric Glands.—Præcardiac glands normal.

Duodenal and Pancreatic Glands.—Normal.

Mesenteric Glands.—The glands in the lower part of the mesentery were enlarged and fused together to form a lobulated yellow mass, measuring about 2½ in. by 2½ in by 1 in.; on section the substance of each gland was found to be converted into a soft caseous pulp. In the upper part of the mesentery the glands were normal in size and appearance, with one exception; this was slightly enlarged and showed in the cortex a yellow patch of softening.

In the mesentery near the ileo-cœcal junction there was a small mass of glands, more or less movable on each other; on section some of the glands were caseous and softened practically throughout, whilst the others showed lesser degrees of caseation. Quite near the junction were two isolated glands; one the size of a pea almost entirely caseous; the other free from caseation.

Colic Glands.—In the mesentery of the ascending colon there was a yellow fluctuating mass, the size of a thrush's egg, around the margins of which were numerous small glands which were normal in appearance; on section the mass was composed of three or four caseous and softened glands which had fused together; near this group there was a pea-sized caseous and softened gland.

On the transverse colon there were two glands close together, the largest the size of a pea; both of these were caseous and softened throughout Other glands along the margin of the colon and the rectal glands appeared normal.

At the root of the meso-colon near the spine there was a group of glands; four of these were about 8 mm. in diameter and showed the substance partly or wholly caseous; two contained each a small nodule; a few others were normal.

Spleen.—The spleen was intimately adherent to the parietal peritoneum and was removed from it only with difficulty; it was much enlarged, measuring 5 by 2½ by about 1 in. in greatest thickness; on its convex surface there was a greyish adherent membrane and two raised firm yellow caseous masses. On section the pulp was closely beset with small irregular soft yellow caseopurulent nodules, here and there aggregated together to form a larger nodule or a cluster, the individuals forming which were separated by a small amount of splenic tissue.

Splenic Lymphatic Glands.—One the size of a pea was caseous and softened practically throughout; another was partly caseous; others were a little enlarged but were not otherwise abnormal.

Liver.—The capsule of the liver on the right side was intimately adherent to the parietal peritoneum, and on removing the organ was completely stripped from the anterior surface of the right lobe. On the posterior surface of the liver there were numerous flattened slightly raised nodules, the majority not larger than 2 mm. in diameter, which resembled the peritoneal nodules; on the left lobe there were some larger nodules, irregular in outline, ranging up to 1 cm. in diameter; these were greyish-white, and caseous in the centre and penetrated varying distances into the substance of the liver. On the anterior surface the capsule that remained showed numerous small nodules, and one larger more raised and more opaque nodule with a connective tissue outgrowth from its surface; in the substance underneath this there was a soft caseous nodule 4 mm. in diameter.

The liver substance was pale and showed on section sparsely scattered translucent tubercles and a few caseous nodules, some of which were bile-stained. A gland in the hilum of the liver showed two or three tubercles. Two glands in the small omentum were enlarged and contained a few soft yellow nodules.

Kidneys.—The kidneys were very pale; the left showed three translucent nodules, the largest the size of a hemp seed; the latter was slightly opaque and yellowish in the centre.

The right kidney showed three nodules on the surface similar to those in the left and two or three small transparent tubercles; the latter were seen with difficulty on account of the pallor of the tissue surrounding them.

On section one small tubercle was seen in the depth of the left kidney and two or three in that of the right.

Suprarenal Bodies.—Normal.

Thorax.

Each pleural cavity contained a small quantity of clear serous fluid.

Lungs.—The lungs were expanded, congested and œdematous; they contained scattered nodules, ranging in size from a millet seed to that of a pea, evenly distributed throughout the parenchyma; the larger ones were firm yellow and caseous throughout, the smaller ones were grey and translucent or showed minute opaque central foci; there were no areas of consolidation.

Bronchial Glands.—On the left side one in the root of the lung contained a soft caseous tubercle; another near the latter, much enlarged, showed no foci of caseation; other bronchial glands were a little enlarged and firmer than normal, but no foci of caseation were seen.

The Ventral Mediastinal Glands on each side were enlarged, firm and contained each a yellow caseous nodule and several small tubercles.

Dorsal Mediastinal Glands.—Normal.

Pleura.—The parietal pleura on the left side showed one pin-head sized tubercle.

On the pleural surface of the diaphragm there were numerous hard flattened tubercles similar to those on the omentum ; they were most numerous on the left side, the largest being about 2 mm. in diameter. Under the pleura covering the diaphragm were three yellow projecting caseous nodules (? tuberculous glands), the largest the size of a kidney bean.

Near the apex of the heart there was a small gland in a state of early caseation.

Heart.—The pericardial sac was filled with clear serous fluid. The heart muscle and valves were normal.

Inguinal and Axillary Glands.—Normal.

The glands along the abdominal aorta were normal. Under the periosteum about the middle of the parietal suture there was a slightly projecting nodule nearly 1 cm. in diameter ; on section this contained soft caseous material ; the bone beneath it was slightly necrosed.

Brain and Meninges.—Normal.

Microscopical Examination (*Smear Preparations*).

Right Tonsil.—Numerous tubercle bacilli.

Liver (*Nodule in Depth*).—A few tubercle bacilli.

Emulsion of Lung.—Numerous tubercle bacilli.

Emulsion of Spleen.—A few tubercle bacilli.

Nodule on Parietal Bone.—Numerous tubercle bacilli.

Animals Inoculated.

A rabbit inoculated intraperitoneally with a small dose of an emulsion made from the lung and killed 76 days later, showed general tuberculosis of the usual type.

A rabbit inoculated intraperitoneally with a small dose of an emulsion made from the spleen and killed 76 days later, showed general tuberculosis, not very severe.

BABOON PASSAGE.

VIRUS B. I.

BABOON 8.

Fed on January 22nd, 1904, with milk of Cow 64 (Table 1A, Volume I.) containing 1,000,000 tubercle bacilli.

Killed.—April 28th, 1904. 97 days later.

Abstract of Post-Mortem Notes.

Ulceration and caseation of both tonsils. Great enlargement and caseation of the pharyngeal, and superficial and deep cervical glands; caseous foci in submaxillary lymphatic glands; one caseous nodule in the right submaxillary salivary gland, and several in the right half of the thyroid. Intestines and mesenteric glands normal. The liver showed several caseating nodules, and a number of miliary tubercles. The spleen contained numerous large softened caseous nodules; the kidneys a few small tubercles. The lungs showed numerous large firm caseous nodules; the bronchial glands caseous foci. There was miliary tuberculosis of the peritoneum, omentum and diaphragm.

A rabbit inoculated intraperitoneally with an emulsion made from a cervical gland was killed, when dying, 88 days after; the post-mortem examination showed general tuberculosis.

A culture was isolated from the cervical gland of Baboon 8 and after cultivation for 533 days was used to feed Baboon 64.

BABOON 64.

Fed with culture from Baboon 8.

Dose.—0·1 milligramme.

Date.—October 13th, 1905.

Died.—January 22nd, 1906. 101 days later.

Clinical History.

The baboon remained lively and well until the end of December. She then became quieter and more irritable and the appetite was not so good. During the first fortnight in January these symptoms became more pronounced and she was obviously losing flesh; the appetite was much diminished.

Towards the end of the experiment she became very emaciated and weak. During the experiment the respirations were never increased and there was no diarrhœa.

Tuberculin Test.—November 24th, 1905, 42 days after feeding. Positive. Rise of temperature 1·7° C.

Post-Mortem Examination.

Body emaciated; no subcutaneous fat.

Both Tonsils were ulcerated and entirely destroyed by caseation; the margins of the ulcers were undermined and the edges congested; the floors were covered with soft caseo-pus, dark coloured on the surface (result of haemorrhage).

Tongue.—Most of the solitary lymphatic follicles at the base of the tongue were caseous and two were ulcerated.

Pharynx.—Normal.

Right Submaxillary Lymphatic Glands.—Normal.

On the right facial vein there were two small caseous tubercles, and on the lower part of the right masseter muscle a caseous gland, the size of a wheat grain.

On the left side there were six enlarged submaxillary glands, the largest the size of a large pea; these on section showed soft caseous nodules, in some replacing almost the whole of the gland substance.

The Left Submaxillary Salivary Gland contained a caseous nodule, about 1·5 mm. in diameter.

The Salivary Gland on the right side was normal.

Retro-Pharyngeal Glands.—Behind each tonsil there was a large irregular mass, the size of a pigeon's egg, consisting of several glands fused together; on section they were all caseous throughout and softened.

Cervical Glands.—In the anterior triangle on the left side and under the insertion of the sterno-mastoid there was a group of enlarged caseous and softened glands; the largest one was the size of a pigeon's egg.

Under the sterno-mastoid in the lower part of the neck there was a chain of enlarged glands, ranging in size from a pea to that of a sparrow's egg; in the posterior triangle under the anterior border of the trapezius there was a gland the size of a sparrow's egg; all these glands were caseous and softened throughout. Two small glands under the middle of the clavicle contained small caseous nodules.

On the right side an upper cervical gland, the size of a French bean, showed caseous areas replacing almost the whole of the substance. A mid cervical gland, slightly enlarged, contained three soft caseous nodules, the largest 3 mm. in diameter. The lower cervical glands on this side were normal.

Abdomen.

The Peritoneal Cavity contained several cubic centimetres of turbid serous fluid.

The Omentum was drawn up and was adherent in several places to the floor of the abdomen and the intestines; it was congested and closely beset with caseous tubercles, the largest 3 mm. in diameter; along the free border the tubercles were closely aggregated together and formed firm masses. The omentum surrounded the spleen which it enclosed in a complete bag; this part of the omentum was also beset with caseous nodules and showed several firm masses composed of aggregated caseous nodules embedded in reddish tissue; the omentum was adherent to the spleen along the hilum and to several projecting caseous nodules.

The Parietal Peritoneum was covered with slightly raised yellowish white caseous tubercles, the largest about 2 mm. in diameter; they were most numerous towards the pelvis and on the diaphragm where they showed a tendency to coalesce.

There were fairly numerous caseous tubercles, up to 2 mm. on the mesentery, and also fairly numerous miliary caseous tubercles on the serous surface of the ileum; on the rest of the intestine they were more sparsely scattered.

Stomach.—Normal.

Small Intestines.—The mucous membrane of the duodenum showed two small ulcers, one with congested margins; in the rest of the intestine there were six typical tuberculous ulcers, majority situated in the ileum; five of these were transverse and ranged from 8 to 10 mm. in length; the margins were thickened and congested and the base contained caseous nodules which projected from the peritoneal surface. The sixth ulcer, situated about three feet from the ileo-cœcal valve, was large and irregular and had congested margins and yellow necrotic base, which was adherent to the peritoneum over the lumbar vertebrae. The margin of the ileo-coecal valve showed an irregular ulcer with congested margins and caseous base.

Large Intestine.—Normal.

Gastric Glands.—A præcardiac gland contained a small caseous nodule.

Mesenteric Glands.—In the lower part of the mesentery there was an irregular nodular mass of enlarged glands, measuring 1½ by 1½ by nearly 1 in.; on section the glands composing the mass were caseous throughout and had thickened fibrous capsules. The glands in the upper part of the mesentery were not much affected; one was slightly enlarged and caseous throughout; one or two others contained discrete tubercles.

Ileo-Colic Glands.—On one side of the junction of the ileum and coecum there was a group of three glands; two were caseous throughout and fused together; the third was œdematous and deeply congested but free from tubercles. On the opposite side two small glands contained caseous tubercles.

Colic Glands.—Two mid-colic glands lying close together were enlarged to the size of a pea; one was partly caseous, the other was caseous throughout. Other colic glands were normal.

Pancreatic Glands.—Around the head of the pancreas there were several enlarged glands, the largest the size of a sparrow's egg; all were caseous and softened throughout. Along the superior border of the pancreas there were several normal glands.

The Spleen contained two large hydatid cysts; one measured 2½ in. by 2½ in. by 2 in.; the other 1½ in. in greatest diameter; only a small amount of splenic tissue remained; this contained a number of caseous nodules, varying much in size, the largest being about the size of a thrush's egg; the majority were flattened out on the surface of the large cyst from which they projected; on section they were soft, yellow, and caseous throughout. Two splenic lymphatic glands contained caseous tubercles.

The Liver.—Was soft and contained five or six hydatid cysts, the largest the size of a hen's egg; scattered throughout the substance were caseous tubercles, with grey margins, mostly about 1 mm. in diameter; a few were larger and measured 2 mm. in diameter.

The Portal Gland was enlarged and showed more than half the substance caseous.

Kidneys.—The right kidney showed on the surface two caseous miliary tubercles; in the depth of the cortex there was a caseous nodule 3 mm. in diameter. In the cortex of the left kidney there was a caseous nodule, 3 mm. in diameter, projecting from the surface.

Suprarenal Bodies.—Normal.

Thorax.

Heart.—Normal.

Pleura.—On the costal pleura there were a few flattened caseous nodules, each about 2 mm. in diameter.

On the pleural surface of the diaphragm, around the margins of the tendon, there was a number of caseous tubercles in several places arranged in clusters and surrounded by reddish granulation tissue; on the pleura covering the muscle of the diaphragm, chiefly on the left side, there was an eruption of grey granules.

Lungs.—In each of the three lobes of the right lung there was a hydatid cyst; they ranged in size from a pigeon's egg to a hen's egg; in the right cephalic lobe there was a smaller cyst; the lungs also contained fairly numerous firm caseous nodules evenly distributed, ranging in size from about 2 up to 8 mm. in diameter; the nodules were caseous throughout with the exception of a narrow grey or greyish red margin.

Bronchial Glands.—A superior bronchial gland on the right side contained two caseous foci. Other bronchial glands appeared normal. The ventral mediastinal glands were enlarged and closely beset with discrete caseous tubercles.

Special Lymphatic Glands.

Axillary and Inguinal Glands.—Normal.

Lumbar Glands.—Two, the size of sparrows' eggs, were caseous throughout; several others contained discrete caseous tubercles.

Iliac Gland.—Normal.

Uterus.—Normal.

Brain and Meninges.—Normal.

Microscopical Examination (Smear Preparations).

Cervical Gland, }
Lung. } A few tubercle bacilli.

Guinea-pig Inoculated.

A guinea-pig inoculated with an emulsion made from the lung died 8 days afterwards, too soon for tuberculosis to have developed.

BABOON PASSAGE.

Virus B. IV.

BABOON 22.

Fed for 25 days with tuberculous milk of Cow 172 (Table IVa, Volume I) from September 9th, 1904, until October 4th, 1904.

Died.—December 8th, 1904. 90 days after the experiment began.

Abstract of Post-Mortem Notes.

Both tonsils were enlarged but not ulcerated; the right showed in the centre a soft caseous mass; the left two caseous foci; the lymphoid follicles at the base of the tongue were enlarged, caseous, and ulcerated. The mucous membrane of the stomach showed six small tuberculous ulcers. The small intestine was severely affected; the upper half was covered with typical tuberculous ulcers with sub-peritoneal tubercles; the lower half was affected chiefly in the last 12 inches, where there were several groups of ulcers in the Peyer's patches. In the first part of the large intestine there were six ulcerated caseous nodules. Pharyngeal, superior cervical, ileo-colic, several colic, and the lumbar glands were greatly enlarged and caseous throughout. The mesenteric glands formed a very large irregularly softened caseous mass adherent in its upper part to the duodenum into which it had ulcerated. The mesentery corresponding to the upper half of the intestine was greatly thickened and tuberculous. Omentum and peritoneum studded with tubercles. Lungs crammed with caseous nodules, the largest the size of a large marble. Bronchial glands only slightly affected. The spleen contained numerous large softened caseous nodules; the liver caseous nodules up to the size of a pea and innumerable minute grey foci. The left kidney contained two caseous nodules, the right one. Diaphragm covered with tuberculous growths chiefly on the right side. One ileo-sacral, two splenic, and two left submaxillary glands contained caseous tubercles; the portal glands were beset with caseous nodules.

Animals Inoculated.

A culture was isolated from a cervical gland and, when it had been in cultivation 292 days, used to inoculate three rabbits and to feed Baboon 62.

No. of Rabbit.	Initial Weight in grammes.	Final Weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
481	1,330	1,020	1·0 mg.	Intraperitoneal	Died 25 days	General tuberculosis of the usual acute type.
482	1,280	1,070	0·1 mg.	Intraperitoneal	32 days (killed accidentally)	General tuberculosis, almost as severe as in Rabbit 481.
480	1,200	1,050	0·01 mg.	Intraperitoneal	Died 37 days	General tuberculosis; lungs packed with caseous coalescing tubercles. Tubercles not so numerous on the peritoneum as in the first two cases.

BABOON 62. (Adult Male).

Fed with culture from Baboon 22.

Dose.—0·1 milligramme.

Date.—September 26th, 1905.

Died.—February 9th, 1906. 136 days after feeding.

Clinical History.

During the first seven weeks his condition was unchanged. About the middle of November he was quieter and more tractable, and towards the end of November he began to have difficulty in breathing, especially after food, due, apparently, to enlargement of the retro-pharyngeal glands.

He then (from about the middle of December) began gradually to lose flesh and strength; the appetite, however, remained undiminished.

The glands in the neck became more and more enlarged and caused greater and greater difficulty in breathing; ordinary quiet respiration was accompanied by a snoring noise, which was very pronounced during sleep and when drinking.

At the end of January he was very thin and weak and moved very little about his cage.

On the day preceding his death there was a hæmorrhagic discharge from the nostrils.

During the experiment there was no increase in the rate of respiration, and no diarrhœa.

At the beginning of the experiment an attempt was made to get a record of the temperature, but was discontinued on account of the animal's uncontrollable struggles.

Post-Mortem Examination.

Tongue.—Normal.

Both Tonsils were slightly enlarged and composed of firm greyish red tissue, containing irregular patches of caseation; there was no ulceration.

Submaxillary Glands.—On the right side three, anterior to the salivary gland, were slightly enlarged and contained from one to three soft caseous nodules, the largest about 3 mm. in diameter. In a gland near the angle of the jaw there were four miliary caseous tubercles. On the left side one gland contained a pea-sized softened caseous nodule.

Submaxillary Salivary Glands.—Normal.

Retro-Pharyngeal Glands.—The pharyngeal glands were very greatly enlarged, and formed on each side a large irregular mass extending from the base of the skull to the level of the larynx. The naso-pharynx was almost completely filled in. The two masses had united in the mid-line in the upper part; the wall of the pharynx here showed a deep necrotic ulcer from which recent hæmorrhage appeared to have taken place. On section the glands on each side were fused together and were caseous and softened throughout.

Cervical Glands.—On the left side in the middle of the neck there was a large soft mass measuring $1\frac{3}{4}$ in. by $\frac{3}{4}$ in. by about $\frac{2}{3}$ in., which on section was found to be composed of several glands fused together; these were all completely caseous and softened. Internal to the carotid artery on the level of the larynx there was a gland the size of a pigeon's egg, which on section was a thin-walled cyst filled with caseo-pus (immediately above this gland were the enlarged pharyngeal glands); between this and the clavicle along the carotid artery there was a chain of small glands which were normal in appearance. Behind the left clavicle there were three small glands beset with soft caseous tubercles and a gland the size of a broad bean, composed of greyish-red translucent tissue closely studded with soft caseous tubercles, up to 1·5 mm. in diameter.

In the middle of the neck on the right side external to the carotid artery there was a large soft gland measuring 1¾ in. by 1 in. by 1 in. which on section was filled with caseo-necrotic masses and caseo-pus; internal to the carotid artery there was a gland the size of a sparrow's egg, which was caseous and softened practically throughout (immediately above this were the enlarged pharyngeal glands).

Behind the outer extremity of the clavicle on the right side there were two glands, each 1 cm. in greatest diameter, filled with caseous and softened nodules which almost completely replaced the gland substance.

Abdomen.

The Peritoneal Cavity contained a small amount of blood-stained fluid.

The Omentum was very closely studded with yellow, caseous, and softened nodules ranging in size from a No. 4 shot to a barley grain ; in many parts of the omentum the nodules were closely aggregated together ; the lower part of the omentum showed a large irregular mass, composed of reddish fibroid tissue, closely beset with caseous softening nodules up to 2 mm. in diameter.

On the meso-colon and serous surface of large intestine there were fairly numerous caseous nodules, similar to those in the omentum. There were similar but less numerous nodules on the mesentery ; these stood up prominently above the surface, and in many places occurred in small clusters.

The Parietal Peritoneum showed chiefly on the right side and on the diaphragm fairly numerous somewhat flattened caseous nodules ; on the left side and in the right lumbar region there was besides an eruption of minute transparent granules.

Stomach.—The mucous membrane showed numerous punctiform hæmorrhages ; otherwise normal.

Small Intestine.—In the early part of the ileum there was a small transverse healing ulcer with congested margins ; in the base there were about half a dozen caseous tubercles which projected from the serous surface. In another part of the ileum there was a caseous tubercle about 1·5 mm. in diameter, which projected beyond both surfaces ; the mucous membrane was not ulcerated.

Large Intestine.—Except where it was adherent to the ileo-colic glands, was normal.

Gastric Glands.—A gland in the lesser curvature of the stomach was enlarged, and contained three soft, yellowish, caseous nodules, up to 3 mm.

Duodenal Glands.—On the first part of the duodenum above the head of the pancreas there were several glands ; one contained a caseous tubercle ; the others were normal. Below the head of the pancreas there was a group of enlarged glands, all of which were caseous and softened throughout.

Mesenteric Glands.—The mesenteric glands were enlarged and fused together to form a smooth fluctuating tumour 2 in. by 2 in. by 1½ in. ; on the surface a few caseous nodules projected from under the peritoneum ; on section the tumour was a very thin-walled cyst filled with caseo-pus.

Ileo-Colic Glands.—The glands on one side of the junction of the ileum and the cœcum formed a large, irregular, caseous and softened mass which was adherent to the cœcum in two places : at one of these places the mass had ulcerated into the lumen of the gut, the mucous membrane showing three separate openings through which caseo-pus could be expressed.

On the opposite side of the junction there were two slightly enlarged glands which contained a few soft caseous nodules.

Colic Glands.—Three showed soft caseous nodules in the cortex ; a few others showed one or two early tubercles.

The Spleen was enlarged and measured 3½in. by 1½in. by 1 ; on the surface there were a few flattened caseous nodules ; the pulp contained very numerous thin-walled nodules filled with yellow tenacious pus (mucoid in character) ; they ranged in size from a shot to a large pea.

In the hilum of the spleen there was a caseous and softened gland, the size of a French bean.

Liver.—On the surface under the capsule there was a moderate number of caseous nodules, with fibrous margins, ranging from 1 to 2 mm. in diameter : in the depth no large nodules were seen, but extremely minute grey tubercles just visible to the eye and a few miliary tubercles with slightly opaque centres.

The Portal Gland was enlarged and contained in the cortex two or three soft caseous nodules.

The Left Kidney showed in the cortex three caseous nodules, 1·5 to 3 mm. in diameter.

The Right Kidney contained one soft caseous nodule 2·5 mm. in diameter.

Suprarenal Bodies.—Normal.

Thorax.

Heart.—The pericardium was universally adherent to the heart, the pericardial sac being obliterated ; the heart muscle and valves were normal.

Lungs.—The left lung showed numerous thin fibrous adhesions to the costal pleura and diaphragm ; the two lobes were intimately adherent.

The right lung was firmly adherent to the diaphragm and showed more recent adhesions to the ribs.

The right upper and lower lobes were crepitant, except for a patch of red hepatisation in the latter, and contained numerous caseous nodules with narrow grey margins, ranging from 1 to 3 mm. in diameter ; in the upper lobe there was besides a caseous nodule 1 cm. in greatest diameter. The right middle lobe was completely consolidated and externally had a dark red colour ; on section it was beset with caseous nodules, which near the root of the lobe were closely aggregated together and much softened.

The left lobes contained numerous caseous nodules similar to those in the right ; there were besides four larger nodules, one measuring 1·5 cm in greatest diameter.

Pleura.—There were no tubercles on the pleura.

Bronchial Glands.—The prætracheo-bronchial glands were enlarged and contained discrete caseous nodules up to 2 mm. in diameter ; one intertrachea-bronchial gland on the right side was much enlarged, œdematous and congested, and contained similar nodules.

Dorsal Mediastinal Glands.—Normal.

Ventral Mediastinal Glands.—Enlarged and very closely beset with discrete soft caseous nodules ranging up to 2 mm. in diameter.

Larynx and Trachea.—Normal.

Special Lymphatic Glands.

Cœliac Glands.—One the size of a swallow's egg, was caseous and softened throughout.

The Lumbar and Iliac Glands were enlarged and contained numerous soft yellow caseous nodules, up to 2·5 mm. in diameter (one right iliac gland was normal).

Inguinal and Axillary Glands.—Normal.

Special Organs.

Testicles.—Normal ; on the tunica vaginalis were several small hard tubercles.

Brain and meninges.—Normal.

Under the periosteum of one of the frontal bones there was a slightly raised red gelatinous nodule, about 1 cm. in diameter, containing soft yellow foci ; this nodule lay in a shallow carious depression in the bone.

Microscopical Examination (*Smear Preparations*).

Left Tonsil.—Fairly numerous T.B.

Spleen (*emulsion*).—A few T.B.

Lung (*emulsion*).—A few T.B.

Nodule under Periosteum of Frontal Bone.—Numerous T.B.

Guinea-pigs Inoculated.

No. 1899, inoculated intraperitoneally with an emulsion made from a lung nodule, died 43 days later of general tuberculosis.

No. 1900, inoculated intraperitoneally with an emulsion made from the spleen, was killed with 1·0 cc. tuberculin and found to have general tuberculosis.

RHESUS MONKEY AND BABOON PASSAGE.

Virus B. I.

MONKEY 76.

Fed on April 7th, 1904, with 0·01 mg. of culture obtained from a supramammary lymphatic gland of Cow 44. *see* Vol. I.

Killed when dying, November 21st, 1905. 228 days after.

Abstract of Post-mortem Notes.

Tonsils and pharynx, normal. Twelve to fifteen small caseous ulcers in small intestine. Enlargement, caseation and softening of the pharyngeal, abdominal and thoracic lymphatic glands. Liver beset with miliary tubercles and softened nodules. Spleen, adherent, greatly enlarged and extensively caseated. Each kidney contained moderately numerous tubercles. The lungs showed numerous miliary tubercles and several large consolidated areas, some far advanced in caseation; in one of the latter there was an irregular cavity. There was tuberculosis of the omentum, peritoneum, pleura and pericardium.

A culture was isolated from the spleen and used to inoculate a series of six rabbits and to feed Baboon 66.

All the rabbits died of general tuberculosis.

BABOON 66.

Fed with culture from Monkey 76.

Dose.—0·1 milligramme.

Date.—September 27th, 1905.

Died.—February 7th, 1906. 133 days after.

Clinical History.

There was no disturbance in the general health until about the middle of January. He was then noticed to be becoming quieter and to be less inclined for food; he also seemed to be losing flesh. He rather quickly became weaker and thinner and died about 17 days after he was first observed to be ill. There was no increase in the respiration, and no diarrhœa during the experiment.

Tuberculin Test.—November 24th, 1905, 58 days after the experiment began. Doubtful. The temperature rose to 39·4° C. (maximum before the inoculation of the tuberculin 38·8 C.) 5 hours after inoculation, and then fell to 38·1° C. at the 12th hour; it gradually rose again and reached 40·7° C. at the 27th hour.

Temperature.—The temperature remained practically normal (maximum 39·2° C.) until January 19th; it then gradually fell to a lower and lower level, and finally no temperature could be recorded with the clinical thermometer.

Post-Mortem.

Body.—Slightly emaciated.

Tongue and Tonsils.—Normal.

Pharynx.—The mucous membrane of the vault of the pharynx showed an oval ulcer, 1 cm. in length, with inverted edges, smooth, undermined margins and congested, uneven floor; the base was fibroid and contained yellow caseous tubercles.

Submaxillary Glands.—Normal.

Retro-Pharyngeal Glands.—On the right side there was a group of three glands, the largest the size of a swallow's egg; these were firm and practically caseous throughout.

On the left side there were four glands, the largest the size of a large pea; two of these were caseous throughout; the third was partly caseous; the fourth contained discrete caseous nodules, irregular in outline.

Cervical Glands.—On the right side in the middle of the neck there was a gland the size of a broad bean which was firm and practically caseous throughout (composed of closely aggregated tubercles); below this there was a gland the size of a French bean closely beset with firm caseous nodules; between the carotid artery and the larynx there were three small glands which were caseous throughout; two small glands under the right clavicle contained caseous tubercles.

On the left side the cervical glands were normal.

Abdomen.

The Omentum was closely studded with caseous nodules with grey margins, ranging in size from a millet seed to that of a wheat grain; they remained discrete except near the spleen, where they were aggregated together into two or three small groups.

The Parietal Peritoneum near the spleen was studded with flattened caseating tubercles up to 1 mm.; there were similar caseous tubercles on the peritoneum covering the diaphragm, and near the spleen a yellow caseous patch about 1 mm. thick; below the spleen the peritoneum was covered with small pearly grey tubercles; on the right side the tubercles were sparsely scattered.

On the mesentery and serous surface of stomach there were a few nodules similar to those in the omentum.

Stomach.—Mucous membrane normal.

Small Intestine.—About 12 inches from the pylorus there was a small circular ulcer with irregular, slightly thickened margins and thin fibroid base in which one or two yellow points were seen. In a Peyer's patch 15 inches from the ileo-cœcal valve there was an oval transverse ulcer 1 cm. in greatest diameter with thick raised irregular margins and caseous base; the peritoneum over them was thickened and darkly pigmented; there were no sub-peritoneal tubercles.

Large Intestine.—Normal.

Mesenteric Glands.—In the upper part of the mesentery there was a large gland, 1 in. in greatest diameter; on section it was caseous throughout and softened in the centre. In the lower part of the mesentery there was a group of three glands, the largest ⅔ in. in diameter; these were also caseous throughout, but not softened. Most of the glands in the middle of the mesentery were normal; some contained small caseous tubercles or small nodules.

The Ileo-Colic Glands.—Were normal with one exception, which contained a small caseous focus.

Colic Glands.—One contained an irregular caseous nodule about 2 mm. in diameter; the rest were normal.

The Spleen.—Was somewhat enlarged and contained about 15 spherical nodules, some of which projected from the surface, majority about 1·5 cm. in diameter; the nodules were yellow and caseous throughout and softened in the centre.

Splenic Lymphatic Glands.—In the hilum of the spleen several lymphatic glands were enlarged and contained discrete caseous tubercles embedded in firm grey tissue.

The Liver.—Was normal in general appearance and showed scattered throughout the substance moderately numerous caseous tubercles ranging in size from 0·5 to 2 mm. in diameter.

The Portal Gland.—Contained three or four caseous tubercles in the cortex.

Kidneys.—Normal.

Suprarenal Bodies.—Normal.

Thorax.

Heart.—Normal.

Pleura.—Costal pleura normal; on the tendon of the diaphragm on the left side there was a yellow granular patch composed of closely aggregated tubercles; elsewhere the diaphragmatic pleura showed scattered caseous tubercles.

The Lungs.—Collapsed normally, and were crepitant throughout. The left lower lobe was adherent to the patch on the diaphragm, the visceral pleura at this point being opaque and thickened.

In the right upper lobe there was one opaque miliary tubercle; in the right middle lobe there were two caseating tubercles, 1 and 2 mm. in diameter respectively; in the right lower lobe there were about half-a-dozen caseous tubercles with grey margins, ranging from 1 to 2·5 mm. in diameter.

The left lobes contained each about nine miliary caseous tubercles.

Bronchial Glands.—One left prætracheo-bronchial gland contained a firm caseous nodule 5 mm. in diameter; another contained a smaller caseous nodule. A right prætracheo-bronchial gland contained a small caseous tubercle. An intertracheo-bronchial gland on the right side showed one caseous tubercle and two on the left side small caseous nodules.

The Dorsal Mediastinal Glands.—Five in number, were enlarged, firm, and caseous throughout.

The Ventral Mediastinal Glands.—Contained a few discrete tubercles.

Lumbar Glands.—Slightly enlarged, showed on section firm caseous tubercles, varying in size.

Iliac Glands.—One on the left side, the size of a pea, was caseous throughout; those on the right side were normal.

Inguinal and Axillary Glands.—Normal.

Brain and Meninges.—Normal.

Testicles.—Normal.

Microscopical Examination (*Smear preparations*).

Spleen - - - -	2 tubercle bacilli seen.
Cervical Gland - -	4 tubercle bacilli seen.

RHESUS MONKEY PASSAGE.

Virus B. IX.

MONKEY 110.

Fed on June 2nd, 1905, with 1·0 mg. of culture derived from the original material of Virus B. IX.

Killed, when dying, September 28th, 1905. 80 days later.

Abstract of Post-Mortem.

Tonsils and glands in neck normal. The stomach showed one small ulcer; the small intestine, 10 typical tuberculous ulcers, the largest 10 × 4 mm.; there were two submucous caseous nodules in the large intestine. The mesenteric glands were caseous throughout, and formed a large fluctuating mass. Almost all the other abdominal lymphatic glands were enlarged and caseous. The spleen contained 7 or 8 caseous nodules, the largest the size of a pea; the liver showed about two dozen caseous tubercles on the surface and there were fairly numerous caseous tubercles in each kidney. The lungs were closely beset with miliary caseous tubercles, and the bronchial glands were caseous. There were two tubercles in the right suprarenal body, two on the omentum, one in the pancreas, and one on the parietal pleura. The cortex of the brain showed about half a dozen caseous nodules.

Animals Inoculated.

A culture was isolated from an iliac gland, and when it had been in cultivation 55 days was inoculated intraperitoneally into two rabbits and subcutaneously into two rhesus monkeys, Nos. 114 and 116. Both rabbits died of general tuberculosis in 22 and 37 days respectively.

RHESUS MONKEY 114.

Subcutaneous.—Inoculation of culture from Monkey 110.

Dose.—0·01 milligramme.

Date.—November 22nd, 1905.

Died.—December 25th, 1905. 33 days after inoculation.

Clinical History.

The monkey was inoculated subcutaneously between the shoulders on November 22, 1905.

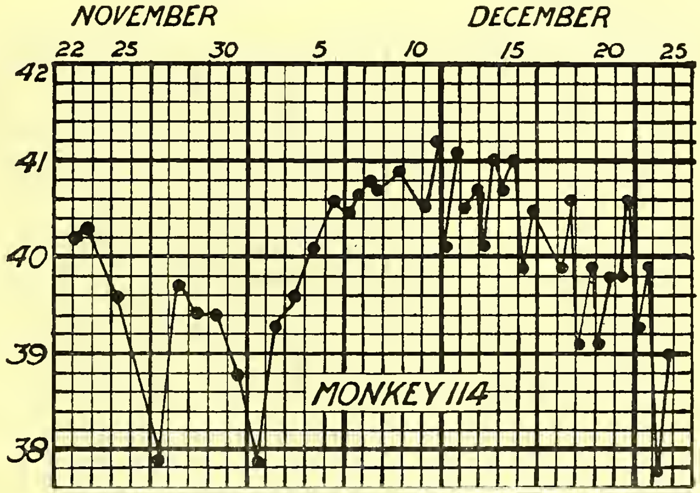
Fifteen days after inoculation there was a rounded swelling, about 1½ in. in diameter, soft and fluctuating in the centre; there was a small opening in the skin. Six days later the swelling was larger and there were three openings in the skin through which yellow pus was discharged.

On December 15 the skin showed an ulcer ½ in. in diameter covered by a scab; removal of this and pressure on the tumour caused thick brownish yellow pus to exude; there were also two small openings covered by scabs. The ulcers enlarged and coalesced and the tumour became much collapsed and appeared to have increased in area.

The axillary glands on both sides became moderately enlarged.

At the end of the third week the monkey was noticed to be unwell; he was thinner and appeared to be losing appetite. The general condition gradually became poorer, and although the appetite was moderate he was obviously losing flesh. On December 23rd he became much worse, and was found dead on the 25th.

Temperature



Post-mortem.

Body.—In poor condition.

Local Lesion.—At the site of inoculation in the middle line of the back, just below the inferior angle of the scapula, there was a slightly irregular oval ulcer, 1 in. × ¾ in.; the margins were thin, reddened, undermined, and partly caseous. The ulcer led into a cavity 2 in. in diameter filled with soft caseous substance; the walls were formed by the muscles of the back, which were infiltrated by caseation.

The Right Axillary Gland.—½ in. long, had the cortex caseous and contained discrete tubercles in the medulla. The gland on the left side was similar.

Thorax.

Parietal Pleura.—Normal.

The right pleural cavity contained an excess of fluid.

Lungs.—The right lung was dark and congested and contained a moderate number of discrete tubercles up to 2·5 mm. in diameter. The largest were caseous with translucent grey margins. On the dorsal surface of the lung there was a fibrinous deposit.

The left lung was pink in colour and contained fewer tubercles up to 2 mm.: they were grey and translucent with opaque centres.

Bronchial Glands.—The right bronchial gland was enlarged and contained soft yellow caseous tubercles up to

1·5 mm.; they were numerous and were arranged round the cortex. The left bronchial gland was small and contained three caseous tubercles. Each prætracheo-bronchial gland contained a single miliary tubercle.

Heart.—In the muscle of the right ventricle there was one pinhead-sized tubercle, and one twice the size in the left. Cavities of the heart normal.

In the dorsal mediastinum on each side of the spine there was a chain of soft caseous lymphatic glands the largest the size of a kidney bean.

Abdomen.

Peritoneum and Omentum.—Normal.

The Spleen was greatly enlarged, measuring 3 in. $1\frac{3}{4}$ in. × $\frac{3}{4}$ in.; it contained numerous tubercles, varying in size from minute grey points up to 3 mm. in diameter. The largest were irregular in outline, opaque and caseous throughout. In the splenic lymphatic gland there were a few discrete tubercles.

The Liver was large and was closely beset with tubercles the smallest minute and grey, the largest up to a pin-head in size, yellowish and caseous.

The Portal Gland was enlarged and the cortex was filled with yellowish tubercles larger than a pinhead.

Kidneys.—In each kidney there were about six scattered grey tubercles with minute opaque centres: on section there were scattered tubercles in the depth. In the right kidney in addition there was a pin head sized yellow tubercle.

Suprarenal Bodies.—In the right suprarenal there was one opaque miliary tubercle.

Lymphatic Glands.

The Mesenteric Glands were not enlarged and in each of these there was a minute opaque tubercle.

In the *Submaxillary, Inguinal, and Left Iliac Glands* there were from one to three pinhead sized caseous tubercles.

Lumbar Glands.—Normal.

Microscopic Examination (Smear Preparation).

Inguinal Gland.—Numerous tubercle bacilli.

Animals Inoculated.

A culture was recovered from an axillary gland and, when it had been in cultivation 27 days, was inoculated subcutaneously into three rabbits and used to feed two monkeys, Nos. 130 and 132.

All the rabbits died of general tuberculosis.

RHESUS MONKEY 116.

Subcutaneous.—Inoculation of culture from Monkey 110.

Dose.—0·01 milligramme.

Date.—November 22nd, 1905.

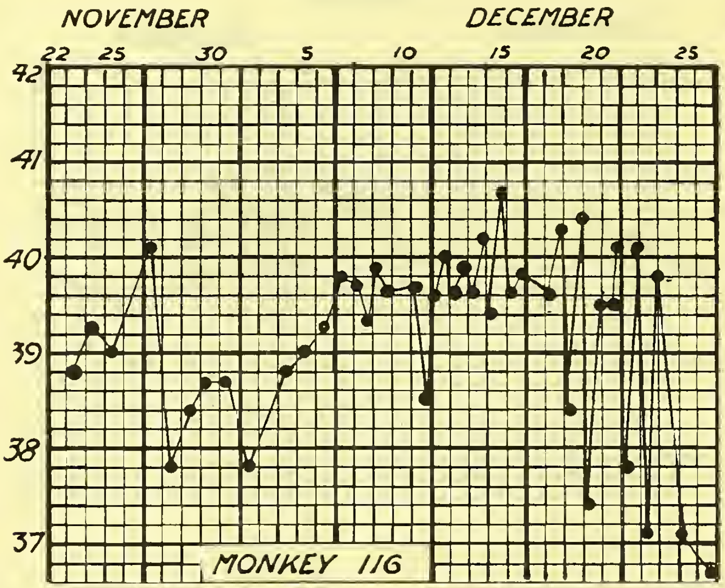
Died.—December 28th, 1905. 36 days after inoculation.

Clinical History.

The monkey was inoculated subcutaneously between the shoulders.

A fortnight after inoculation there was a slightly raised soft swelling measuring nearly 1in. in diameter; on the 23rd day the swelling measured $1\frac{3}{4}$ in. by $1\frac{1}{4}$ in. and subsequently there was a little further increase in size. A fortnight after inoculation the axillary glands were felt to be slightly enlarged; during the rest of the experiment they underwent little further enlargement. Up to December 22 there was no change in the general condition, except that latterly the animal was quieter; then illness (rough coat, depression and anorexia,) rapidly supervened and the animal soon became collapsed and died.

Temperature.



Post-mortem.

Body emaciated.

Local Lesion.—At the site of inoculation, in the mid line of the back, there was a soft fluctuating tumour ($2\frac{1}{2}$ in. by $1\frac{1}{4}$ in.) situated between the skin and muscle. On section it showed a fibro-caseous wall filled with yellow caseo-pus.

The Axillary Glands on both sides were enlarged and contained nodules filled with soft caseo-pus.

Cervical Glands.—The right inferior cervical gland was enlarged and contained pinhead yellow tubercles; the left was small and appeared normal.

Abdomen.

The Spleen, little if at all enlarged ($1\frac{1}{2}$ in. by $\frac{3}{4}$ in. by $\frac{1}{2}$ in) contained numerous tubercles varying in size, the smallest minute and grey, the largest up to 3 mm. in diameter, with soft yellow caseous centres. Those under the capsule projected slightly.

Liver.—The liver contained numerous minute grey foci just visible and a moderate number of large tubercles varying in diameter up to 3 mm. The largest of these had thin fibrous capsules and were completely softened in the centre.

The Portal Gland was a little enlarged and contained a soft yellow nodule.

The Kidneys and *Suprarenal Capsules* appeared normal.

Thorax.

Heart.—Normal.

Pleura.—The right pleural cavity contained a little fluid, and the right lung was more collapsed than the left. On the left side under the pleura at the junction of the eighth rib with the spine was a pea-sized softened yellow nodule.

Lungs.—In the lungs were sparsely scattered grey translucent tubercles up to 2 mm. in diameter, the largest of which showed minute soft yellow centres.

Bronchial Glands.—The bronchial glands were normal.

Various Lymphatic Glands.

Lumbar and Iliac Glands.—Normal.

Sternal glands were a little enlarged and contained pinhead sized caseous tubercles.

There was a pinhead sized caseous tubercle in each sub-maxillary gland, and in each of two mesenteric glands and in a left inguinal gland. Right inguinal glands normal.

Microscopical Examination.

Axillary gland showed a moderate number of T.B.

RHESUS MONKEY 130.

Fed with culture obtained from an axillary gland of Monkey 116.

Dose—0·01 milligramme.

Date—January 24th, 1906.

Killed, when dying, April 20th, 1906. 86 days after being fed.

Clinical History.

Signs of ill-health, rough coat and depression were first noticed on March 31st, 1906.

On April 16th the monkey was obviously ill and appeared to be losing flesh; there was occasional cough but no increase in respiration.

The animal rapidly became worse and lost appetite; it was killed in a dying condition on April 20th, 1906. 86 days after the experiment began.

Tuberculin Test.—April 10th, 1906. 76 days after the experiment began. Positive. Rise of temperature 1·1° C.

Temperature.

The temperature was slightly raised from February 21st to February 26th, maximum 39·7° C.; and again from March 29th until the end of the experiment, maximum 40°C.

Post-mortem Examination.

Body.—Condition, moderate.

Tongue, Tonsils and Pharynx.—Normal.

Submaxillary, Retro-Pharyngeal and Cervical Glands.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Stomach.—Normal.

Small Intestine.—Normal.

Large Intestine.—Normal.

Mesenteric and Colic Glands.—Normal.

One Ileo-colic Gland contained a grey miliary tubercle, with a caseous centre.

A Cœliac Gland contained a similar tubercle.

A Gland on the head of the pancreas contained two miliary caseating tubercles.

The Spleen was enlarged and showed on the surface numerous projecting yellow nodules, the largest about 8 mm. in diameter; the nodules were thin-walled and filled with caseo-pus; on section similar nodules were seen in the interior of the spleen.

Splenic Lymphatic Glands.—Normal.

The Liver was normal in general appearance; in the substance just under the capsule, there were three or four tubercles, the largest about 1 mm. in diameter with caseous centres and grey margins. On section two or three small tubercles were seen in the depth.

The Portal Gland appeared normal.

The Left Kidney showed in the cortex a grey tubercle with a slightly opaque centre. In the right kidney there was a grey miliary tubercle with no central opacity.

Suprarenal Bodies.—Normal.

Thorax.

Heart.—The pericardial sac contained a small quantity of clear serous fluid; heart muscle normal.

Lungs.—The right middle lobe and an accessory right middle lobe were much enlarged and filled the lower part of the right pleural cavity; the former was firmly adherent to the chest wall in front and laterally and less firmly to the upper and lower lobes; on section the middle lobe was composed partly of grey translucent tissue, closely beset with irregular caseous patches and tubercles, and partly of firm yellow caseated tissue, showing here and there small islands of grey translucent tissue; the accessory middle lobe was almost uniformly yellow and caseous; there was no sign of softening.

The right lower lobe was crepitant in the dorsal part and consolidated near the root; the solid portions were composed of firm translucent grey tissue in a less advanced stage of caseation than the middle lobes; the crepitant part contained a few discrete caseating nodules.

The right upper lobe contained about eight nodules, similar to those in the left lobes, and a small patch composed of grey caseating tubercles closely aggregated together.

The left lobes were dark red, collapsed and quite airless; they contained altogether about two dozen nodules, ranging in size from a pin's head to that of a pea; on section the nodules were firm yellow and caseous in the centre, grey and translucent around the margins.

Bronchial Glands.—On the left side two prætracheo bronchial glands were affected; one contained a caseous nodule, 2 mm. in diameter; another a small caseous tubercle; one intertracheo-bronchial gland on this side contained a miliary caseous tubercle.

On the right side the glands were moderately enlarged; two prætracheo-bronchial glands were beset with caseous tubercles, here and there becoming confluent; one intertracheo-bronchial gland, the size of a broad bean, was composed of irregular yellow caseous areas, some softened, separated by a small amount of translucent grey tissue.

Ventral Mediastinal Glands.—One on the right side, the size of a French bean, was beset with discrete caseous tubercles, irregular in outline, ranging from a mere point to 1·5 mm. in diameter; another contained a miliary caseous tubercle; the rest were normal.

Vertebral Glands.—The glands in the intercostal spaces near the vertebral column were on the right side all enlarged; one in the third space showed three caseating tubercles and one in the ninth one minute tubercle; in the rest there were no opaque foci. On the left side the glands were normal.

Parietal Pleura.—The pleura, where it was adherent to the right middle lobe, was roughened and shaggy and showed two loosely attached caseous nodules, the largest 3 mm. in diameter; there were two similar nodules in the upper part of the chest and about half-a-dozen miliary caseating tubercles.

Lumbar and Iliac Glands.—Normal.

Inguinal and Axillary Glands.—Normal.

Brain and Meninges.—Normal.

RHESUS MONKEY 132.

Fed with culture obtained from an axillary gland of Monkey 116.

Date—January 24th, 1906.

Dose—0·0001 milligramme.

Died—June 7th, 1906. 134 days after the experiment began.

Clinical History.

The monkey showed no obvious signs of ill-health until about the middle of May; the coat was then noticed to be rough and he appeared to be losing flesh; he gradually became perceptibly thinner and lost appetite and was found dead on June 7, 1906, 134 days after the experiment began; the respirations were not noticeably increased and there was no diarrhœa.

Tuberculin Test.—10th April, 1906, 76 days after the experiment began. Positive. Rise of temperature, 1·2°C. Dose of tuberculin 2·0 cubic centimetres.

Temperature.—The temperature from the beginning of the experiment until 19th April, was normal, with exception of a slight rise, to 39·8° C. on 3rd April, 1906. From April 16th to May 10th there was a period of slight pyrexia (maximum, 39·0° C.); then after a slight fall the temperature rose, and from May 15th to May 25th was sustained at a fairly high level (maximum 40·8° C.); subsequently it assumed a lower level, falling finally below 37° C.

Post-mortem Examination.

Tongue, Tonsils and Pharynx.—Normal.

Submental, Submaxillary, Retropharyngeal and Cervical Glands.—Normal.

Abdomen.

The Omentum contained a few small transparent bodies which were probably early tubercles.

Stomach and Intestines.—Normal.

Gastric Glands.—A gland in the lesser curvature of the stomach near the pylorus contained a pin-head sized caseous tubercle.

Mesenteric Glands.—One mesenteric gland, the size of a large pea, was filled with yellow tenacious caseo-pus; other mesenteric glands were normal.

Ileo-colic and Colic Glands.—Normal.

Spleen.—The spleen showed on the surface half-a-dozen projecting nodules, the largest the size of a large pea; three of these were very prominent, projecting more than half their diameter from the surface; the others were completely surrounded by spleen pulp, and caused only slight projections; in the depth of the spleen three more nodules, the largest the size of a pea, were seen; all the nodules were caseous and softened throughout.

The gastro-splenic omentum was adherent to two of the projecting nodules, and showed between them about half-a-dozen millet-seed-sized caseous tubercles.

The lymphatic glands in the hilum of the spleen were perhaps slightly enlarged, but were not otherwise abnormal.

Liver.—The liver was normal in general appearance; on the surface under the capsule there were three caseous nodules, ranging from 1 to 2·5 mm. in diameter, the largest of which was softened; in the depth of the liver substance there was a nodule, 2 mm. in diameter, with a grey margin and bile-stained caseous centre.

In the Small Omentum, between the head of the pancreas and the liver, there were three enlarged glands; one of these was caseous almost throughout; the others contained soft yellow caseous nodules up to 3 mm. in diameter.

Kidneys and Suprarenal Bodies.—Normal.

Thorax.

Lungs.—The left lower lobe was enlarged, and filled the greater part of the left half of the thorax; it was practically everywhere adherent to the chest wall by adhesions which were recent and easily torn through, except at the apex, where the two layers of pleura were united more firmly; the right lung was free from adhesions.

On removal of the lungs the left lower lobe was firm, heavy and quite airless; on the dorsal surface of the apex there was a patch of thickening; externally the ventral thinner parts of the lobe had a greyish red translucent appearance, whilst the rest of the lobe showed a yellowish mottling; on section the whole of the lobe was in a condition of caseous pneumonia; the dorsal portions were almost uniformly caseous and softened, and in the midst of some of the caseous areas small cavities had formed; in the thin ventral portions a small amount of translucent greyish red tissue separated the caseous areas.

The upper lobes were adherent to the lower lobe, and were firm, red and airless except for a narrow margin along the dorsal border; these lobes contained a few caseous tubercles.

The upper and lower lobes were crepitant and congested on the dorsal surfaces; they contained sparsely scattered shotty caseous tubercles, with grey margins, ranging from about 1 to 2 mm. in diameter.

One of the small middle lobes was enlarged, firm and airless except at the extreme tip; on section it was advanced in caseation and beginning to soften. Another small middle lobe showed in the ventral part a caseous mass, 1·5 cm. in greatest diameter, with translucent margins; extending from this mass to the root of the lung on the internal surface there was a reddish consolidated patch, which showed on section grey foci (? early caseation).

Pleura.—There were no tubercles on the pleura.

Heart and Pericardium.—Normal.

Bronchial Glands.—The glands on the right side were enlarged, congested and œdematous; one below the bronchus contained a minute caseous point; in the others there was no sign of caseation.

On the left side a prætracheo-bronchial gland, the size of a thrush's egg, was caseous and softened throughout; another slightly smaller gland in the root of the lung on the dorsal surface of the bronchus was also caseous throughout; an intertracheo-bronchial gland on this side was large, firm and congested, and contained one pin-head-sized caseous tubercle. Some glands along the trachea and a few ventral to the arch of the aorta were enlarged, congested, but were not otherwise abnormal.

Dorsal Mediastinal Glands.—Normal.

Axillary Glands.—One on the left side contained a softened yellow caseous nodule, 2 mm. in diameter; the others were normal.

Inguinal, Lumbar and Iliac Glands.—Normal.

Brain and Meninges.—Normal.

Testicles.—Normal.

Microscopical Examination (Smear Preparation).

Bronchial Gland.—Numerous tubercle bacilli.

Spleen.—Numerous tubercle bacilli.

Liver.—Numerous tubercle bacilli.

Axillary Gland.—Numerous tubercle bacilli.

RHESUS MONKEY 152.

Subcutaneous—Inoculation of an emulsion made with the spleen of Rhesus Monkey 132.

Date—June 7th, 1906.

Died—September 26th, 1906.

Duration of Experiment—111 days.

Clinical History.

The animal was inoculated in the subcutaneous tissue of the back on June 7th, 1906. After three weeks there was a small thickening in the subcutaneous tissue at the seat of inoculation and an opening in the skin over it from which issued a blood-stained purulent discharge; the axillary glands were not perceptibly enlarged. The tumour increased in size and the skin over it became ulcerated in several places. The glands in the axillae enlarged, the skin over them became adherent and finally ulcerated.

Until three weeks before its death the monkey was lively and ate well; it showed no sign of ill-health except that it increased very little in size. After the first manifestation of illness its condition became progressively and rapidly worse.

Post-mortem Examination.

Body.—In poor condition.

Local Lesion.—In the subcutaneous tissues over the lower part of the right scapula there was a small softened caseous tumour, the skin over which was pierced by a number of small openings through which caseo-pus could be made to exude; the hair was covered with masses of dried secretion. In the tissues around the tumour there was a number of discrete yellow softened nodules up to a hempseed in size.

Axillary Glands.—In the right axilla there was a group of three enlarged glands, the largest the size of a pigeon's egg, which on section were composed of creamy yellow caseo-pus and a fibrous wall varying in thickness; the skin over two of the glands was adherent and ulcerated, the floors of the ulcers being formed by the dried caseous contents of the gland.

In the left axilla there were two enlarged glands, one the size of a large pea, the other a little smaller, both of which were caseous and softened practically throughout.

Cervical Glands.—Normal.

Thorax.

Lungs.—The lungs were crepitant and collapsed normally, they contained moderately numerous nodules varying greatly in size, ranging from about the size of a pin's head up to about 1 cm. in diameter; most of the larger nodules had caseous and softened centres and relatively thick fibrous margins, but a few were composed of a firm greyish tissue containing purulent foci (resembling muco-pus); the smallest nodules were firm, grey, and had opaque yellowish centres.

Bronchial Glands.—Four (two on each side) were much enlarged and varied in size from 1 to 2 cm. in diameter; on section they were thin-walled cysts filled with soft yellow tenacious caseo-pus; the other bronchial glands were enlarged and one contained a miliary caseous tubercle.

Mediastinal Glands.—One of the ventral mediastinal glands on the right side, the size of a large pea, was caseous and softened throughout.

Vertebral Glands. On the right side of the vertebral column opposite the heads of the eighth to tenth ribs there was a prominent thin-walled tumour, the size of a thrush's egg, filled with thick caseo-pus; the right caudal lobe was closely adherent to the capsule of the tumour; other vertebral glands were normal.

Pleura.—Costal pleura, normal. The tendon of the diaphragm showed about half a dozen flattened nodules with fibrous margins and soft caseous centres, ranging in size from a millet seed to a hempseed.

Heart.—Normal.

Abdomen.

Omentum.—The omentum contained six nodules ranging in size from a pin's head to a hempseed; the larger ones were caseous and softened in the centre; the smaller ones were translucent.

Spleen.—The spleen contained two pea-sized yellow caseous and softened nodules with thin fibrous capsules and about a dozen smaller caseous nodules ranging in size from that of a pin's head to that of a hempseed. In the hilum of the spleen there were three enlarged glands, all of which were caseous and softened throughout.

Liver.—Sparsely scattered over the surface of the liver were grey translucent foci, the largest 1 mm. in diameter; these extended nearly their own diameter into the substance and did not project from the surface. In the substance there was a number of thin walled cysts, containing a greenish mucoid material, ranging in size from that of a pea downwards; there were besides a few caseous tubercles with fibrous margins.

Closely adherent to the head of the pancreas there was a mass of fused glands (probably including the portal) the size of a pigeon's egg; all the glands were entirely converted into thick yellow caseo-pus, which in each case was surrounded by a thin fibrous capsule.

Kidneys.—The left kidney showed in the depth of the cortex a minute grey tubercle. The right kidney showed a similar tubercle on the surface and three in the depth.

Suprarenal Bodies.—The right suprarenal body contained a firm yellow caseous nodule, 5 mm. in diameter. The left suprarenal body was normal.

Small Intestine.—The wall of the small intestine showed about eighteen thickened caseating patches, the largest of which extended transversely around nearly the whole circumference of the gut; the mucous membrane over them was ulcerated, the larger areas showing several separate ulcers. On the serous surface of the larger areas and in the mesentery there was a well-marked tuberculous lymphangitis.

Large Intestine.—The mucous membrane of the large intestine showed eight ulcers, with congested margins, the largest 1 cm. in greatest diameter; the bases of these ulcers were thickened (in some cases considerably) and fibroid and beset with soft caseous foci.

Mesenteric Glands.—The mesenteric glands were greatly enlarged and formed two large fluctuating tumours, one in the upper part, nodular in outline, about the size of a pigeon's egg, and one in the lower part about the size of a hen's egg; the two tumours were united together at the middle of the mesentery; the duodenum lay in close contact with the upper mass, but could be easily separated from it. On section all the glands composing the tumours were converted into thin-walled cysts filled with thick creamy caseo-pus; when the latter was washed away under a stream of water nothing was left but a multilocular cyst wall.

Ileo-colic Glands.—The glands on each side of the junction of the ileum and cœcum were enlarged and resembled on section the mesenteric glands.

Colic Glands.—Several of the colic glands (eight to ten in number) were enlarged, up to 1 cm. in diameter, caseous and softened throughout.

Ilio-sacral Glands.—Two ilio-sacral glands, the size of large peas, were yellow, caseous and softened throughout.

Lumbar Glands.—A left lumbar gland contained a millet-seed sized, soft, caseous tubercle.

Iliac Glands.—A left iliac gland, the size of a pea, was caseous and softened thoughout; the right was normal.

Inguinal Glands.—One on the left side resembled the iliac; the rest were normal.

Tongue, Tonsils, Pharynx, Submaxillary and Pharyngeal Lymphatic Glands.—Normal.

Microscopical Examination.

Lung Nodule.—Numerous tubercle bacilli.

Splenic Lymphatic Gland.—Very numerous tubercle bacilli.

MONKEY AND BABOON PASSAGE.

Virus B. IX.

MANGABEY MONKEY 4.

Fed on June 2nd, 1905, with 1·0 mg. of culture derived from the original material of Virus B. IX.

Killed.—August 25th, 1905. 84 days later.

Abstract of Post-mortem.

Tonsils and neck glands normal. The small intestine showed five tuberculous ulcers. The mesenteric glands were much enlarged, caseous throughout and softened. The lungs and spleen contained numerous caseous nodules, the largest the size of a pea. The liver was closely beset with caseous tubercles and there were several caseous tubercles in each kindey. One bronchial gland showed caseous areas; the rest were normal. The dorsal mediastinal and lumbar glands were extensively caseated, and there were areas of caseation in the portal, splenic and pancreatic glands. There were some tubercles on the omentum and pleural surface of the diaphragm.

A culture was isolated from a lumbar gland and used to feed Mandrill Baboon 68.

MANDRILL BABOON 68.

Fed with culture from Mangabey Monkey 4.

Dose.—0·1 milligramme.

Date.—October 13th, 1905.

Died.—November 28th, 1905. 46 days later.

Clinical History.

Illness was first noticed on November 13th, 1905; he was much quieter and seemed to be losing appetite. These symptoms continued and he became thinner and lost strength. On November 20th he had a fit in the morning, suddenly losing power over his limbs; there was no loss of consciousness and only occasional slight twitchings of the legs; after a little while the symptoms abated and he was able to walk about and climb on to his perch. The fits recurred on every subsequent morning until death, and occasionally in the afternoon as well.

Post-mortem Examination.

Body in poor condition.

Tongue and Pharynx.—Normal.

Tonsils.—In one of the follicles of the right there was a little purulent material. (No tubercle bacilli found; numerous organisms stained blue.)

Left Tonsil.—Normal.

Right Retro-Pharyngeal Gland, about the size of a sparrow's egg, contained viscid tenacious yellow purulent material almost completely replacing the gland substance. (Tubercle bacilli found.)

Left Retro-Pharyngeal Gland.—Normal.

Submaxillary and Cervical Glands.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Stomach and Small Intestines.—Normal.

Large Intestines; mucous membrane congested; no ulceration.

Gastric, Mesenteric and Colic Glands.—Normal.

Spleen, Liver, Kidneys and Suprarenal Bodies.—Normal.

Thorax.

Heart and Pleura.—Normal.

Lungs and Bronchial Glands.—Normal.

The Muscles all over the body showed moderately numerous cysticerci (? taenia saginata); the tongue, the heart, and the wall of the stomach each contained one, and the diaphragm two.

The Brain contained numerous similar cysts; some were completely embedded in the cortical substance; others projected varying distances from the surface; the latter by their pressure had caused shallow cup-shaped hollows on the internal surface of the vault of the skull.

Microscopical Examination. (*Smear Preparations.*)

Retro-Pharyngeal Gland. Tubercle bacilli scanty.

Animals Inoculated.

Guinea-pig 1785, inoculated with an emulsion made from the spleen, died in 36 days of general tuberculosis.

A culture was isolated from a pharyngeal gland and, after cultivation on artificial media for 57 days, was used to inoculate three rabbits subcutaneously and to feed Monkeys 126 and 128.

The rabbits all died of general tuberculosis.

MONKEY 126.

Fed with culture obtained from the right retro-pharyngeal gland of Baboon 68.

Date—January 24th, 1906.

Dose—0·01 milligramme.

Died—May 24th, 1906. 120 days after the experiment began.

Clinical History.

The monkey remained well until about the end of April; he was then noticed to be quieter, and to have a "staring" coat.

He gradually became more depressed, and, although the appetite remained moderately good, slowly lost flesh and strength; there was no increase in the rate of respiration, and no diarrhœa.

Tuberculin Test.—April 10th, 1906, 76 days after the experiment began. Positive. Rise of temperature 1·9°C. Maximum 41·1°C. 3 hours after injection. Dose of tuberculin 2·0 cubic centimetres.

Temperature.—During the first twelve weeks the temperature was normal, varying from 39·3°C. to 38°C.; on April 17th it began to rise and for twelve days there was a period of moderately high pyrexia (maximum 41°C.); the temperature then became irregular and began gradually to fall; the daily fluctuations were often more than 2°C.; during the last few days the temperature was subnormal and on two occasions fell to 36°C.

Post-mortem Examination.

Body.—Slightly emaciated.

Tongue, Tonsils and Pharynx.—Normal.

Submaxillary, Pharnygeal and Cervical Glands.—Normal.

Abdomen.

The Peritoneal Cavity.—Contained about 15 cubic centimetres of clear fluid.

The Omentum.—Contained four yellow nodules, irregular in outline, each the size of hemp-seeds, which on section were entirely caseous and beginning to soften; there were, besides, four caseous tubercles, the size of millet seeds.

The Parietal Peritoneum was normal.

Stomach.—Normal.

Small Intestine.—One foot from the pylorus the wall of the small intestine showed a ring-like thickening, 13 mm. in width, which completely girdled the bowel and slightly constricted the lumen; the mucous surface was congested in patches and showed several irregular ulcers; on section all the coats of the intestine were found to be destroyed by caseation; the mesentery corresponding to the thickened intestine was almost completely obliterated, the intestine lying in close contact with the enlarged mesenteric glands; the lacteal vessels on the thickened intestine near the mesenteric attachment were opaque and yellow.

About a foot further along the intestine there was a raised area, 7 mm. in diameter, ulcerated in the centre; the submucous tissue beneath this area was caseous. Near the latter there was a small submucous caseous nodule, which was not ulcerated. Elsewhere the intestine was normal.

Large Intestine.—Normal.

Gastric Glands.—A gland near the cardiac end of the stomach was normal; one on the lesser curvature near the pylorus contained two soft caseous nodules, the largest 3 mm. in diameter.

Mesenteric Glands.—The mesenteric glands were much enlarged and formed a mass measuring 2¼ × 2 × 1¼ in.; this mass was composed of two thin walled, closely united, fluctuating swellings, one on each side of the mesentery; these swellings were smooth and shiny externally, showing a yellow mottling under the peritoneum, and were filled with soft creamy caseo-pus.

At the upper extremity of this mass, connected with the duodenum, were two glands, each 1 cm. in diameter, which were caseous and softened throughout.

The Ileo-Colic Glands.—Were œdematous, but were not otherwise abnormal.

Colic Glands.—Normal.

Thorax.

Lungs.—The lungs were expanded and filled the chest; they were somewhat emphysematous around the margins. The left lung was everywhere adherent to the chest wall; the adhesions were comparatively recent, except at one place posteriorly, where there was a tough adhesion not easily torn through; the right lung was free from adhesions.

The lobes of the left lung were adherent by recent adhesions. The right lobes were more firmly adherent together, and showed on the ventral and lateral surfaces a thin organised layer of lymph with a pitted or honeycombed surface.

The lung substance was closely beset with firm yellow nodules, ranging in size from rather less than 1 mm. up to 15 by 10 mm. in diameter; the great majority of the nodules, however, measured from 2 to 3 mm. in diameter; on section the nodules were homogeneously caseous throughout; several, particularly the larger ones, were surrounded by reddish patches of consolidation, otherwise the lung tissue between the nodules was crepitant.

Bronchial Glands.—The intertracheo-bronchial glands on each side were much enlarged, their substance being extensively replaced by softening caseous nodules, up to 4 mm. in diameter.

A Left Prœtracheo-bronchial Gland, the size of a kidney bean, was partly caseous.

One on the right side contained one soft caseous nodule. A ventral mediastinal gland contained one pinhead-sized caseous tubercle.

Costal Pleura.—Normal.

On the Diaphragmatic Pleura there was some hypertrophy of the fringes around the margins of the tendon.

The Pericardial Sac.—Contained a small quantity of clear serous fluid.

Heart Muscle and Valves.—Normal.

Abdomen.

Spleen.—Not apparently enlarged, showed on the surface a few small flattened tubercles; under the capsule about a dozen nodules were seen ranging from 1·5 to 8 mm. in diameter; the larger ones projected considerably from the surface and were yellow, caseous, and softened throughout (one of these was adherent to the peritoneum covering the kidney); the smaller ones were also caseous but firmer, and the capsule over some of them showed filmy grey expansions. On section there were two or three caseous nodules in the depth of the spleen.

One Splenic Lymphatic Gland.—Contained two soft caseous nodules, the largest 2 mm. in diameter.

Liver.—Normal in general appearance, contained scattered yellow caseous and softened nodules with thin fibrous capsules, ranging from 2 to 5 mm. in diameter twelve were counted on the surface, and there were a few in the depth, one larger (8 mm.) than the rest and very softened. At the bottom of the fissure between the right and left lobes there was a projecting yellow caseous nodule, 1 c.m in diameter.

The Portal Gland.—Appeared normal.

The Left Kidney.—Showed in the cortex five yellow caseous nodules, 2 to 3 mm. in diameter; four of these projected slightly from the surface. There were, besides, 3 or 4 caseous tubercles the largest the size of a pin's head.

The Right Kidney.—Contained two caseous nodules, the largest 3 mm. in diameter, and one small grey tubercle with an opaque centre.

The Right Suprarenal Body.—Showed in the cortex a softened caseous nodule 2.5 mm. in diameter.

Left Suprarenal Body.—Normal.

Lumbar Glands.—The lumbar glands on the left side, three in number, were much enlarged, caseous, and softened throughout; two were fused together and formed a mass the size of a filbert. On the right side two glands were slightly enlarged and practically caseous throughout.

Iliac Glands.—Normal.

Axillary and Inguinal Glands.—Normal.

Testicles.—Normal.

Brain.—Normal.

On the under surface of the dura mater, covering one of the cerebral hemispheres, there was a soft caseous tubercle.

Microscopical Examination (Smear Preparation).

Lung.—Numerous tubercle bacilli.

Spleen.—Numerous tubercle bacilli.

Dura Mater.—Numerous tubercle bacilli.

RHESUS MONKEY 128.

Fed with culture obtained from the right retro-pharyngeal gland of Baboon 68.

Date—January 24th, 1906.

Dose—0·0001 milligramme.

Killed—July 12th, 1906. 169 days after.

Clinial History.

The monkey remained well during the experiment. Weight at death, 4530 grammes.

Tuberculin Test.—April 10th, 1906, negative.

Post-mortem Examination.

There was no sign of tuberculosis in any of the organs or glands.

The Lungs showed under the pleura a few thin walled cysts containing the *Pneumonyssus griffithi*.

LEMUR PASSAGE.

Virus B. IX.

LEMUR 2.

Fed on June 2nd, 1905, with 1·0 mg. of culture derived from the original material of Virus B. IX.

Killed.—July 18th, 1905. 46 days later.

Abstract of Post-mortem.

There were sixteen ulcers in the small intestine and several ulcers in the cœcum ; the glands in the neck, the mesenteric and colic glands were enlarged and caseous ; the lungs contained four, the spleen twenty-four to thirty, caseous nodules ; the liver showed scattered greyish white tubercles ; the kidneys a few doubtful foci ; the portal glands and a bronchial gland were tuberculous.

A culture was recovered from the mesenteric gland and inoculated into three rabbits and two lemurs (Nos. 10 and 12).

All the rabbits died of general tuberculosis of the usual type.

LEMUR 12.

Subcutaneous.—Inoculation of culture from Lemur 2.

Dose.—1·0 milligramme.

Date.—November 24th, 1905.

Killed, moribund. December 17th, 1905. 23 days after inoculation.

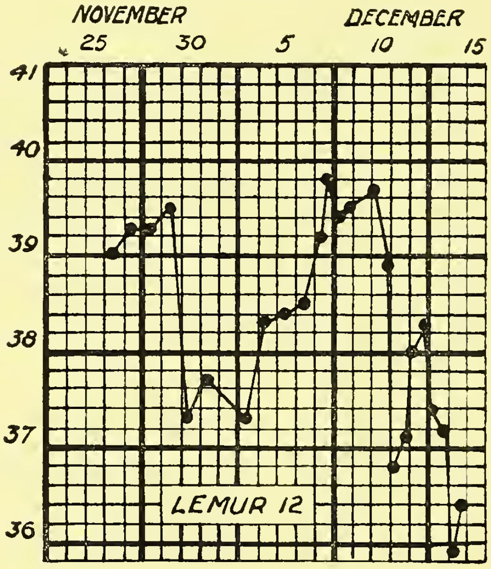
Clinical History.

The animal was inoculated subcutaneously between the shoulders on November 24th, 1905.

A fortnight after inoculation there was a hemispherical fluctuating swelling about 1¼ inches in diameter ; a week later this had increased in size and the axillary glands on the left side were felt to be enlarged ; on the right side enlarged glands could not be detected.

There was no obvious change in the general condition of the animal until December 16th, 22 days after inoculation, when it was found in the morning unconscious and collapsed ; it remained in this state until the following morning and appeared to be dying ; it was thereafter chloroformed.

Temperature.



Post-mortem Examination.

Body in fair condition ; moderate amount of subcutaneous and abdominal fat.

Local Lesion.—At the seat of inoculation between the shoulders (more on the left than on the right side) there was a hemispherical cystic swelling about 1½ in. in diameter and 1 in. in depth ; on section the tumour was a thin-walled cyst filled with chocolate-coloured pus ; the internal surface of the cyst was yellow.

Axillary Glands.—In the left axilla there was a smooth fluctuating mass 1½ in. by ¾ in. by ½ in. composed of two or three glands fused together ; on section these were converted into cysts filled with light brownish pus.

On the right side there was a gland the size of a sparrow's egg which contained a small cavity filled with pus.

Cervical Glands.—Left inferior cervical enlarged, no foci of necrosis seen. Other cervical glands normal.

Omentum and Peritoneum.—Normal.

Spleen.—(?) Enlarged, showed on the surface a grey speckled appearance ; on section Malphigian bodies conspicuous, no tubercles visible.

Liver.—Pale ; no tubercles seen.

Portal Gland.—Normal.

Kidneys and Suprarenal Bodies.—Normal.

Heart and Pleura.—Normal.

Lungs and Bronchial Glands.—Normal.

Tonsils, Pharynx, Intestines, and Mesenteric Glands.—Normal.

Microscopical Examination (Smear Preparation).

Spleen.—Moderately numerous tubercle bacilli.

A guinea-pig inoculated from the spleen died too early for the development of tuberculous lesions.

LEMUR 10.

Subcutaneous.—Inoculation of culture from Lemur 2.

Dose.—0·01 milligramme.

Date.—November 24th, 1905.

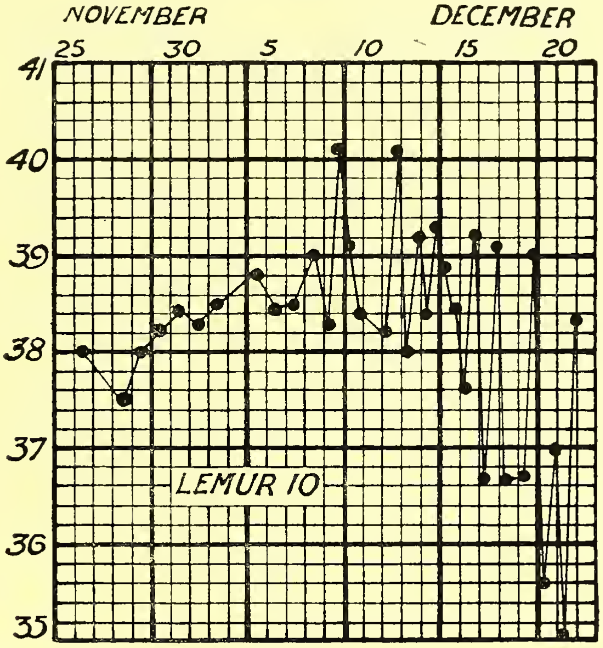
Died.—December 24th, 1905. 30 days after inoculation.

Clinical History.

The animal was inoculated subcutaneously between the shoulders on November 24th, 1905. A fortnight after inoculation there was a prominent fluctuating swelling about $\frac{3}{4}$ in. in diameter; this increased in size, and on December 15th, 3 weeks after inoculation measured $1\frac{1}{4}$ in. in diameter; the skin on this date showed a small opening which discharged thin yellow pus. Subsequently the tumour increased a little in size; and the small opening became covered with dried secretion and only occasionally discharged pus. During the first three weeks there was no obvious enlargement of the axillary glands; at the end of the first month a gland in the right axilla was felt to be enlarged.

The general condition was not changed until towards the end of the experiment, when the animal suddenly became collapsed. During the last three days of life no temperature was recorded with the clinical thermometer.

Temperature.



Post-mortem Examination.

Body.—In fair condition. There was a large amount of fat in the omentum, covering completely the intestines, and also in the lumbar regions

Local Lesion.—At the seat of inoculation in the mid line of the back there was a soft tumour immediately under the skin (measuring 2 in. by $1\frac{1}{2}$ in. by $\frac{1}{2}$ in.). On section it was composed of soft granulation tissue with a cavity just beneath the skin containing clear serous fluid.

The Right Axillary Gland contained discrete caseous foci in the cortex; minute grey foci were seen in the left gland.

Cervical Gland on the right side was enlarged and in part softened without typical caseation. The corresponding gland on the left side was smaller and showed part of the cortex greyish white and firmer.

Abdomen.

The Spleen was a little enlarged and contained about fifteen discrete greyish miliary tubercles.

Liver.—On the surface of the liver nine grey submiliary tubercles could be seen.

Kidneys and Suprarenal Bodies appeared normal.

Thorax.

Heart.—Normal.

The Pleural Cavities were free from fluid.

Lungs.—The lungs contained two minute grey points of a doubtful nature.

Bronchial Glands.—Normal.

Lumbar, Iliac, Mesenteric, and Sternal Glands.—Normal.

DOG PASSAGES.

First Passage with Virus B. IV.

DOG 54.

A collodion capsule, containing an emulsion of culture derived from the original material of Virus B. IV. was put into the peritoneal cavity of the dog on May 30th, 1905.

The culture used was 21 days old, twenty-eighth generation, and was grown on ox serum.

The dog was killed on October 9th, 1905. 142 days after. During the last 6 to 8 weeks of life the dog gradually lost flesh, and when killed was very thin

Post-Mortem Examination.

Body.—Emaciated.

The Peritoneal Cavity.—Contained from 20 to 30 cubic centimetres of brownish turbid fluid.

The glass part of the capsule was found free in the peritoneal cavity; it was filled with whitish flocculent pus.

In the gastro-splenic omentum there was a firm mass the size of a small hen's egg; it lay between the stomach and the tail of the spleen, the latter partly encircling it; on section it was composed of fatty and fibrous tissue, showing here and there some suspicious yellow spots, but no definite caseation or necrosis.

In the centre of the mass were the remains of the collodion part of the capsule.

The Mesentery.—Showed a few small grey tubercles in the centre of some of the areas between the vessels. The surface of portions of the small intestine appeared to be covered with a thin transparent adherent layer: this layer did not cover the surface uniformly, but in several places was seen as small filmy islands.

Parietal Peritoneum.—Normal.

The Peritoneum.—Covering the diaphragm was thickened and showed dense white fibrous tissue containing faint yellow striæ.

The Liver.—Was adherent to the diaphragm and showed a patchy thickening of the capsule: on section the substance was normal.

The Portal Gland.—Seemed a little enlarged, but on section was not obviously tuberculous.

The Spleen.—Was enlarged and contained near the hilum several soft dirty yellowish nodules, up to a large pea in size: these appeared to have developed as the result of direct extension from the tumour.

Kidneys.—Each kidney showed in the cortex numerous tubercles ranging in size from 1 to 1·5 or more millimetres in diameter; many of the tubercles were elongated in a radial direction; they were grey and had minute opaque yellow centres.

The Lungs and Bronchial Glands.—Appeared normal.

The Ventral Mediastinal Glands.—Were enlarged and firmer than normal; on section there was no definite caseation, but from the cut surface a pus-like fluid could be scraped.

Mesenteric Glands and other Lymphatic Glands.—Were normal.

Microscopical Examination (Smear Preparations.)

Scraping from Tumour.—Numerous T.B., mostly in clumps.

Fluid from Glass Tube.—Numerous T.B.

Ventral Mediastinal Gland.—Numerous T.B.

Spleen Nodule.—A few T.B.

Mesenteric Gland.—No T.B.

Guinea-Pigs Inoculated.

No. 1736 was inoculated intraperitoneally with some of the contents of the glass part of the capsule and died in 54 days of general tuberculosis.

No. 1737, inoculated intraperitoneally with an emulsion made from the tumour, died of tuberculosis in 17 days.

DOG 82.

Fed with culture derived from Dog 54.

Dose.—32·5 milligrammes.

Date.—December 15th, 1905.

Killed.—March 16th, 1906. 91 days after.

Post-Mortem Examination.

Body.—In good condition.

Tongue, Pharynx and Tonsils.—Normal.

Submaxillary, Pharyngeal and Cervical Lymphatic Glands.—Normal.

Intestines, Large and Small.—Normal.

Mesenteric and Colic Glands.—Normal.

Spleen.—Normal.

Liver.—Normal.

Portal Gland.—Normal.

Kidneys.—Each kidney showed in the cortex numerous tubercles, ranging in size from about 0·5 to 1·5 mm. in diameter; the large tubercles were greyish and translucent and had in most cases minute opaque centres; the smaller tubercles were spherical, pearly grey, of greyish-white, and when cut into seemed to have narrow grey margins and soft whitish centres (these bodies were not apparently of a tuberculous nature).

The Lungs.—Were crepitant throughout; in one cephalic lobe a small firm nodule was felt; this on section had a reddish colour and was not, apparently, of a tuberculous nature; with this exception the lungs were normal.

Bronchial and Mediastinal Glands.—Normal.

All other Organs and Glands.—Were normal.

Microscopical Examination (Smear Preparations.)

Kidney Tubercle.—No T.B. (Preparation showed a large amount of fatty material.)

Lung Tubercle.—No T.B.

Guinea-pig Inoculated.

Guinea-pig 1935, inoculated with an emulsion made from the kidney, remained healthy.

DOG 92. (Age 6 to 12 months.)

Fed with culture from Dog 54.

Dose.—About 25 milligrammes.

Date.—January 22nd, 1906.

Killed.—March 27th, 1906. 64 days after.

Post-Mortem Examination.

Body.—In good condition.

Tonsils and Pharynx.—Normal.

Submaxillary Glands.—A gland at the left angle of the jaw contained a firm nodule, about 5 mm. in diameter, composed of greyish-red tissue and yellow streaks of necrosis; the gland on the right side was normal.

Pharyngeal Glands and Cervical Glands.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Stomach.—Normal.

Small Intestine.—Mucous membrane normal.

Large Intestine.—Normal.

Mesenteric Glands.—Normal in size; under the peritoneum covering one of the glands there was a doubtful yellow focus; on section nothing abnormal was seen in the substance of the gland (? fat in subperitoneal tissue); a piece of this gland was inoculated into a guinea-pig. Other mesenteric glands were normal.

Ileo-colic and Colic Glands.—Normal.

Liver.—Normal.

Spleen.—Normal.

Suprarenal Bodies.—Normal.

Kidneys.—The left kidney showed in the cortex on the surface a grey tubercle, about 1 mm. in diameter, with a minute opaque central point.

The left kidney contained a similar but smaller tubercle.

Thorax.

Heart and Pleura.—Normal.

The Lungs.—Contained from 10 to 12 hard caseous nodules, ranging in size from a little over 1 to 2·5 mm. in diameter; the nodules were somewhat irregular in outline and had grey translucent margins.

Bronchial and Mediastinal Glands.—Appeared normal.

All other lymphatic glands were normal in size and appearance.

Testicles.—Normal.

Microscopical Examination (Smear Preparations).

Lung Nodules (three examined).—In each a few T.B. were found.

Guinea-pigs Inoculated.

No. 1955, inoculated intraperitoneally with emulsion from mesenteric gland remained healthy.

No. 1954 inoculated intraperitoneally with emulsion from a lung nodule died in 18 days of tuberculosis.

SECOND PASSAGE WITH VIRUS B. IV.

DOG 56.

Age.—About six months.

A collodion capsule, containing an emulsion of culture derived from the mesenteric gland of Chimpanzee 8, was put into the peritoneal cavity of the dog on June 8th, 1905. The sub-culture used was 15 days old, sixth generation.

Killed when ill and emaciated, August 23rd, 1905. 76 days after the experiment.

Post-Mortem Examination.

Body.—Very emaciated.

The Peritoneal Cavity.—Was filled with yellow serous fluid containing a few small flakes of lymph.

In the Great Omentum.—There was an elastic tumour the size of a large hen's egg, firmly adherent to the floor of the abdomen along the mid-line; it was adherent also to the mesentery and to several coils of the small intestine, but from these it could with care be separated. On section the tumour was composed of soft granulation tissue showing many yellow areas of necrosis; the latter had a spongy fibrous texture, and from them turbid puriform fluid could be expressed. In the centre of the tumour the capsule lay embedded, the collodion part was brittle and partially disintegrated; the glass portion of the capsule was filled with turbid purulent fluid.

The serous surface of the mesentery was roughened and finely granular.

The Parietal Peritoneum.—In the lateral regions was profusely covered with tuberculous granulations; a thin layer of yellow lymph covered the peritoneal surface of the diaphragm.

The Spleen.—Was not enlarged; the pulp was pale and firm; on the surface were a few flakes of lymph.

Liver.—Capsule covered with a thin grey fibrinous membrane, thicker and yellower in patches; substance softer and paler than normal; no tubercles seen.

The Portal Gland.—Was much enlarged and showed in the cortex yellow patches of necrosis from which puriform fluid could be squeezed.

Kidneys.—Normal.

Lungs.—Were crepitant and closely beset with hard grey tubercles, the largest the size of a pin's head.

Bronchial and Mediastinal Glands.—Not obviously affected.

Mesenteric Glands and other Lymphatic Glands.—Were normal to the eye.

Microscopical Examination (Smear Preparations).

Tumour (necrotic patch) }
Fluid from glass tube } T.B. extremely numerous.
Portal Gland.—T.B. very numerous.

Guinea-pigs Inoculated.

No. 1647 was inoculated subcutaneously with some of the turbid fluid from the capsule.

No. 1648 was inoculated intraperitoneally with an emulsion made from the abdominal tumour.

Both guinea-pigs died, the first in 37 days, the second in 91 days, of general tuberculosis.

DOG 74. (Adult Retriever.)

Subcutaneous.—Inoculation of culture from Dog 56.

Dose.—10 milligrammes.

Date.—October 13th, 1905.

Killed.—December 10th, 1905. 63 days after inoculation.

Clinical History.

A tumour formed under the skin of the abdomen, which ulcerated. The inguinal and axillary glands enlarged; the skin over them became adherent and then ulcerated. 38 days after inoculation there was a discharging ulcer at the seat of inoculation, an ulcer in the groin and one in the left axilla; the left hind leg was drawn up, movement evidently causing pain; later the left fore-leg also became contracted.

Post-Mortem Examination.

Condition.—Good.

Local Lesion.—In the skin of the abdomen on the left side near the root of a nipple there was a small sinus discharging serous fluid; on section this was found to communicate with a space in the subcutaneous tissues, measuring 2 in. in length and 1 in. in breadth; the walls were in contact and were lined with soft pinkish granulation tissue.

In the left groin there was a linear cicatrix with a small sinus in the centre; pressure caused thin pus to exude from the latter; the sinus terminated at the left inguinal gland and was lined with granulation tissue discharging pus.

The Left Inguinal Gland.—Was indurated, but was otherwise normal to the eye.

In the Left Axillary Region.—There were two small openings in the skin in the centre of small cicatrices; on removal of the skin these were seen to communicate with a large cavity in the axilla lying under the pectoral muscle and extending dorsally as far as the scapula; the cavity was lined with soft granulation tissue and contained a small amount of pus.

The Left Axillary Gland.—Was about twice the size of the right; on section it appeared normal.

Spleen, Liver and Portal Gland.—Normal.

Kidneys.—Each kidney showed in the cortex on the surface a few opaque greyish white tubercles, the largest about 1·5 mm. in diameter; on section the latter were slightly elongated in a radial direction.

Thorax.

Lungs.—The lungs were crepitant and contained fairly numerous evenly distributed, hard grey tubercles, mostly about 1 mm. in diameter; the tubercles showed no sign of caseation.

Bronchial Glands.—Normal.

Heart and Pleura.—Normal.

Mesenteric, Iliac and other Peripheral Lymphatic Glands.—Normal.

Miscroscopical Examination.

Scraping from sinus in groin.—One tubercle bacillus.
Scraping from cavity in axilla.—No tubercle bacilli.

Guinea-pigs Inoculated.

No. 1809, inoculated intraperitoneally with a scraping from the wall of the cavity in the axilla, died in 9 days of an acute infection.

No. 1808, inoculated intraperitoneally with an emulsion from a bronchial gland, was killed 42 days later and showed general tuberculosis.

DOG 116. (Puppy.)

Fed with culture from Guinea-pig 1808, inoculated with an emulsion from the bronchial gland of Dog 74.

Date—March 29th, 1906.

Dose—10 milligrammes.

Killed—July 5th, 1906, 98 days after the experiment began.

Clinical History.

The dog gradually lost appetite, and at the end of the first month had become very emaciated; its appetite then improved, and it began slowly to put on flesh; when killed it was in moderately good health.

Tuberculin Test.—May 10th, 1906. 42 days after the experiment began. Negative. Dose of tuberculin, 5·0 cc.

Post-Mortem Examination.

Tonsils and Pharynx.—Appeared normal.

Submaxillary Glands.—Normal.

Left Retro-Pharyngeal Gland.—Was enlarged and indurated; on section it showed at one extremity a large greyish white somewhat translucent patch containing irregular opaque whitish areas of caseation, some of which were beginning to soften; the cortex of the rest of the gland showed similar but smaller patches; one of these was greyish white and not caseous.

Just posterior to this gland was a small gland (cervical), the substance of which was almost completely replaced by a large greyish white patch, in the midst of which was an irregular opaque white caseous mass beginning to soften. Other cervical glands on this side were normal.

Right Retro-Pharyngeal Glands.—Showed patches in the cortex similar to those in the left pharyngeal; they replaced about half the substance of the gland.

Right Cervical Glands.—Normal.

Parotid Glands.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Intestines.—Scattered throughout the whole length of the small intestine in the submucous tissue were fairly numerous yellow foci, largest less than a millet seed in size, which could just be felt between the finger and thumb (smears from two of these showed no tubercle bacilli but numerous other organisms stained blue). All the Peyer's patches were normal and there was no ulceration anywhere.

Large Intestine.—Normal.

Gastric Glands.—Normal.

Mesenteric Glands.—The mesenteric glands seemed a little enlarged. The glands in the anterior part of the mesentery were normal except for one or two small grey foci. Passing backwards, the glands became more and more severely affected. Those in the middle of the mesentery showed around the cortices sharply defined greyish-white semi-opaque areas, varying much in size, the larger ones containing opaque white friable caseous patches or streaks. The terminal mesenteric gland and the ileo-colic glands showed almost the whole of the peripheral part of the cortex composed of greyish-white caseating tissue.

Colic and Rectal Glands.—Normal.

Spleen.—Was a little soft but not apparently enlarged; on the surface there was a small group of pearly white granules; on section the Malpighian bodies were conspicuous but there were no foci of necrosis.

Liver.—Was normal in general appearance, and showed minute grey foci sparsely scattered throughout; there were besides a grey tubercle the size of a millet seed and an opaque white tubercle about 0·5 mm. in diameter (tubercle bacilli found).

Portal Gland.—Normal.

Kidneys and Suprarenal Bodies.—Normal.

Thorax.

The lungs collapsed normally and showed no patches of consolidation; they contained, evenly distributed throughout, moderately numerous firm grey nodules ranging in size from a millet seed to that of a small pea; most of the nodules were opaque and caseous in the centre; three of the larger nodules were greyish red and showed small cavities in their interior containing a small quantity of semi-purulent material.

The bronchial glands were distinctly enlarged; one on the left side showed in the cortex an opaque pinhead-sized caseous focus; an intertracheo-bronchial gland contained a minute yellow point.

Heart and Pleura.—Normal.

Prepectoral, Prescapular, and Inguinal Glands.—Normal.

Iliac and Lumbar Glands.—Normal.

Microscopical Examination (Smear Preparations).

Left Tonsil.—No tubercle bacilli.

Mesenteric Gland.—Numerous tubercle bacilli.

Liver (large grey tubercle).—No tubercle bacilli.

Liver (white tubercle).—Seven tubercle bacilli.

Intestine (two smears).—No tubercle bacilli.

Lung (two nodules).—Numerous tubercle bacilli.

Animals Inoculated.

Guinea-pigs 2137 and 2138 inoculated intraperitoneally with emulsions made from lung nodules became tuberculous.

VIRUS B. IV. PASSAGE.

DOG 18.

Fed on August 23rd, 1904, with an emulsion made from the organs of Guinea-pigs 1148 and 1150, inoculated with milk of Cow 164.

Dose—10·15 million tubercle bacilli.

Killed—January 5th, 1905, 135 days later.

Post-Mortem Examination.

Two mesenteric glands were enlarged and tuberculous, and there were a few doubtful tubercles in the lungs.

A culture was isolated from a mesenteric gland and used to inoculate a series of rabbits (*see* page 104) and to feed Dog 132.

DOG 132.

Fed with culture obtained from Dog 18.

Date—June 1st, 1906.

Dose—50 milligrammes.

Initial Weight—7470 grammes. Final weight, 8,900 grammes.

Killed—August 17th, 1906, 77 days later.

Post-Mortem Examination.

Pharynx and Tonsils.—Normal.

Pharyngeal Glands.—The left pharyngeal gland contained a single small caseous focus; the right was normal.

Submaxillary and Cervical Glands.—Normal.

Intestines.—Normal.

Mesenteric Glands.—All the mesenteric glands with the exception of two in the anterior part of the mesentery contained small irregular patches and foci of caseation; these were situated in the cortex just beneath the capsule.

Ileo-Colic and Colic Glands.—Normal.

Spleen.—Normal.

Liver.—Normal.

Portal Gland.—Normal.

Kidneys.—Normal

Lungs.—In the left lung there was a grey miliary tubercle with a yellow caseous centre and also a minute slightly opaque grey tubercle; the rest of the lung was normal.

Bronchial Glands.—One on the right side contained an opaque caseous focus. One on the left side contained an irregular caseous tubercle.

Heart and Pleura.—Normal.

Microscopical Examination (Smear Preparations).

Mesenteric Gland.—A few tubercle bacilli.

Bronchial Gland.—No tubercle bacilli.

PASSAGE WITH VIRUS B. IX.

DOG 60. (Puppy.)

Fed on July 10th, 1905, with 1 mg. of culture derived from the original material of Virus B. IX.

Killed.—October 30th, 1905, 112 days later.

Abstract of Post-mortem Examination.

Tonsils and Intestines.—Normal.

The Pharyngeal, and Ileo-colic Glands contained a few discrete opaque white slightly gritty caseous tubercles; three or four mesenteric glands contained dense irregular caseous nodules, up to about 2 mm. in diameter.

There was no sign of tuberculosis in the internal organs.

A culture was isolated from a mesenteric gland nodule and inoculated into a series of rabbits and Dog 84 (by feeding).

All the rabbits died of general tuberculosis.

DOG 84. (Puppy.)

Fed with culture derived from a mesenteric gland of Dog 60.

Dose.—50·0 milligrammes.

Date.—January 2nd, 1906.

Killed.—March 16th, 1906, 73 days later.

Post-Mortem Examination.

Tonsils and Pharynx.—Normal.

Submaxillary, Pharyngeal and Cervical Glands.—Normal.

Small Intestine.—Most of the Peyer's patches showed a number of minute yellow points in the substance, but were not otherwise abnormal.

Large Intestine.—Normal.

Mesenteric and Colic Glands.—Normal.

Spleen, Liver, Kidneys and Suprarenal Bodies.—Normal.

Portal and Cœliac Glands.—Normal.

Heart and Pleura.—Normal.

Lungs.—Normal in general appearance, contained scattered hard grey miliary tubercles, many of which showed a minute opaque point in the centre.

Bronchial Glands.—Normal.

Microscopical Examination (Smear Preparations).

Peyer's patch.—2 T.B.
Lung tubercle.—No T.B.

Guinea-pig Inoculated.

A guinea-pig (No. 1936) inoculated intraperitoneally with an emulsion made from the lung, died in 40 days of general tuberculosis.

DOG 130.

Subcutaneous (partly intraperitoneal) inoculation of culture, 7th generation, 21 days old, isolated direct from the mesenteric gland of Dog 60.

Date.—May 17th, 1906.

Dose.—48 milligrammes.

Weight.—Initial weight 4,570 grammes. Final weight 4,500 grammes.

Died.—August 10th, 1906, 85 days after inoculation.

Post-Mortem Examination.

Local Lesion.—In the skin of the abdomen in the middle line there was an oval ulcer measuring 1 in. by 1¾ ins. It was perfectly clean and was in process of healing. At the posterior extremity the edge was undermined forming a sinus lined with granulation tissue which led down to a left inguinal gland.

In the skin of the left inguinal region there were two small ulcers with granulating sinuses leading to the glands and on the right side one ulcer and sinus. The subcutaneous tissues on both sides were œdematous.

The inguinal glands on both sides were a little enlarged and the cortices were moist and yellow in colour.

Thorax.

Heart and Pleura.—Normal.

The Lungs were congested and œdematous, but floated in water; the substance had a grey translucent appearance.

Bronchial Glands.—Normal.

Ventral Mediastinal Glands.—Slightly enlarged and œdematous.

Abdomen.

The Peritoneal Cavity.—Was distended with a large quantity of clear serous fluid.

The Omentum was thickened by a network of soft grey translucent tissue.

The Mesentery and Meso-colon showed a few scattered minute translucent tubercles.

Spleen.—Appeared normal.

Liver.—The substance of the liver was infiltrated with grey translucent tissue forming a close network.

The Portal Gland was enlarged and was distinctly yellow and necrotic throughout.

The Kidneys contained scattered grey foci in the cortices: on section some of these extended radially through the cortex.

Suprarenal Bodies.—Normal.

Lymphatic Glands.

Axillary, Submaxillary and Cervical Glands.—Œdematous but otherwise normal in appearance.

Mesenteric Glands were œdematous and had a uniform yellow appearance.

Iliac Glands on both sides were œdematous and the left contained a yellow necrotic area.

Microscopical Examination (Smear Preparations).

Liver, Kidney, Spleen, Omentum, Mesenteric Gland, Right Inguinal Gland.—All contained numerous tubercle bacilli.

Lung.—Showed a moderate number of tubercle bacilli, mainly in clumps and cells.

PASSAGE WITH VIRUS B. IX.

DOG 50. (Adult.)

Fed on June 2nd, 1905, with 1 mg. of culture derived from the original material of Virus B. IX.

Killed.—September 21st, 1905, 111 days later.

Abstract of Post-Mortem Examination.

Tonsils and Intestines.—Normal.

Neck Glands and Abdominal Lymphatic Glands.—Normal.

Lungs.—The left cephalic lobe was consolidated and showed a small cavity near the apex containing a small quantity of pus; the left bronchial gland was a little enlarged.

Each kidney contained a few opaque white spherical tubercles, the size of small pins' heads (not tuberculous).

Animals Inoculated.

Two guinea-pigs were inoculated, one No. 1708 with some of the pus from the cavity, the other No. 1707 with an emulsion made from the consolidated lung; both became tuberculous and cultures were isolated from each.

A series of rabbits and a calf No. 374 were inoculated with the culture isolated from Guinea-pig 1708, and two dogs were inoculated, one subcutaneously, the other by the mouth, each with 10 mg. of the culture obtained from Guinea-pig 1707.

Abstracts of the post-mortem notes of the rabbits are given on page 177.

CALF 374.

Subcutaneous.—Inoculation of culture, third generation, 20 days old, derived from the lung of Dog 50, through Guinea-pig 1708.

Date.—January 2nd, 1906.

Dose.—50 milligrammes.

Weight.—1 cwt. 2 qrs.

Killed.—April 3rd, 1906, 91 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on 2nd January, 1906.

A fortnight after inoculation there was a prominent firm rounded swelling, 3½ inches in diameter; the prescapular gland measured about 4 inches in long diameter.

There was no further increase in size, but during the latter part of the experiment the tumour diminished a little and began to soften.

The prescapular gland also diminished a little in size and became very hard and nodular.

About seven weeks after inoculation an enlarged prepectoral gland was felt.

There was no disturbance in the general health of the calf during the experiment.

Tuberculin Test.—March 23rd, 1906. Eighty days after inoculation. Positive. Rise of temperature, 2·4°C.

Weights.

There was a steady gain in weight during the experiment:—

January 2nd, 1906 -	-	1 cwt.	2 qrs.	0 lbs.
January 25th, 1906 -	-	1 cwt.	2 qrs.	11 lbs.
February 6th, 1906 -	-	1 cwt.	2 qrs.	27 lbs.
February 21st, 1906 -	-	1 cwt.	3 qrs.	22 lbs.
March 20th, 1906 -	-	2 cwts.	1 qr.	4 lbs.
April 3rd, 1906 -	-	2 cwts.	1 qr.	25 lbs.
Gain in weight -	-	—	3 qrs.	25 lbs.
Rate of gain per week	—			8·4 lbs.

Temperature.

The temperature remained practically normal throughout the experiment.

Post-Mortem Examination.

Carcass in good condition.

Local Lesion.—At the seat of inoculation on the left side of the neck there was a hemispherical tumour, measuring 3 in. by 3 in. by 1¼ in., composed of a mass of dense yellow caseated tissue, slightly gritty around the margins, surrounded by a zone of fibrous tissue about 3 mm. in greatest thickness; under the skin the mass was separated from its capsule by a layer of thick caseo-pus, but elsewhere was firmly adherent; the fibrous capsule was adherent to the skin, which was thickened, and to the muscles.

The Left Prescapular Gland measured 3¼ in. by 2 in. by 1¼ in., and was very hard and irregular in outline; attached to the convex surface there was a short fibrous cord, the thickness of a crow quill, with caseo-calcareous centre; on section the gland was composed partly of dense gritty caseous masses and partly of fibro-calcareo-caseous tissue; on the convex surface the capsule was greatly thickened, measuring at one place 1 cm. in thickness.

Right Prescapular Gland.—Normal.

Prepectoral Glands.—On the left side the spherical gland was enlarged and contained an irregular calcareo-caseous mass, the size of a kidney-bean, with a fibrous margin; other prepectoral glands on this side were normal.

Right Prepectoral Glands.—Normal.

Cervical Glands.—A gland in the middle of the neck on the left side, the side of a broad bean, showed about half its substance calcareo-caseous. Other cervical glands on this side as well as those on the right side were normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Spleen.—Weight, 20 ounces, normal on the surface and on section.

Liver.—Normal.

Gall-Bladder.—Normal.

Portal Glands.—One contained a number of yellowish-white foci, not perceptibly gritty; another gland contained three or four small very gritty tubercles; in a third gland there was one gritty tubercle.

Left Kidney.—Showed on the surface a minute greyish white focus.

Right Kidney.—Normal.

Suprarenal Bodies.—Normal.

Thorax.

Heart and Pleura.—Normal.

Lungs.—The lungs were normal in general appearance. In the left caudal lobe under the pleura three grey miliary tubercles were seen; one of these had a calcareous centre; besides these there were a few minute transparent points (? tubercles); in the rest of the lung one or two grey points similar to the latter were seen. No tubercles were seen on section.

The Bronchial and Dorsal Mediastinal Glands.—Contained scattered yellow calcareous tubercles, the largest the size of a pin's head.

Infra-tracheal and Ventral Mediastinal Glands.—Normal.

Larynx and Trachea.—Normal.

Tongue, Tonsils and Pharynx.—Normal.

Intestines.—Normal.

Urinary Bladder.—Normal.

Testicles.—Normal.

Thyroid Body.—Normal.

Eyes.—Normal.

Special Lymphatic Glands.

One Coeliac Gland.—Contained a small yellow slightly gritty tubercle.

An Ileo-Colic Gland.—Showed in the cortex two irregular yellow calcareous tubercles.

The Mesenteric, Colic, Gastric, Precrural, Popliteal, Gluteal, Pudic, Iliac, Ilio-Sacral, Lumbar, Renal, Sub-Maxillary, Left Pharyngeal, Mastoid and Axillary.—Were normal.

The Right Pharyngeal Gland.—Contained one small irregular calcareous tubercle.

Microscopical Examination.

Prescapular Gland.—Moderate number of tubercle bacilli.

Pharyngeal Gland. *Portal Gland.* *Coeliac Gland.* *Lung.*	No tubercle bacilli.

Animals Inoculated.

Guinea-pig 1959, inoculated with an emulsion made from the spleen, died in 37 days and showed general tuberculosis.

Calf 394 was inoculated subcutaneously with 20·5 c.c. of an emulsion made from the prescapular gland.

CALF 394.

Subcutaneous inoculation of an emulsion made from the prescapular gland of Calf 374.

Dose.—83·7 million tubercle bacilli.

Date.—April 3rd, 1906.

Weight.—1 cwt. Age, 8 weeks.

Killed.—June 6th, 1906. 64 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on April 3rd, 1906.

A week later a slight indefinite subcutaneous thickening, measuring about 2 by 1, was felt; the prescapular gland was very slightly enlarged.

The thickening gradually diminished and at last became imperceptible and the gland resumed its normal size.

The calf during the whole of the experiment suffered from diarrhœa which was especially severe in the last month.

The temperature remained practically normal throughout (maximum 39·6°C.)

Tuberculin Test.—May 31st, 1906. 58 days after inoculation.—Negative.

Dose of tuberculin about 1 cc.

Weights.

During the first three weeks there was a gain in weight of 2 lbs.; in the last 43 days there was a loss of 7 lbs.

April 3rd and 18th, 1906 -	1 cwt.	0 qrs.	0 lbs.
April 24th, 1906 - -	1 cwt.	0 qrs.	2 lbs.
May 15th, 1906 - -	0 cwt.	3 qrs.	27 lbs.
June 6th, 1906 - -	0 cwt.	3 qrs.	23 lbs.
Loss in Weight - -	0 cwt.	0 qrs.	5 lbs.

Post-Mortem Examination.

Condition.—Moderate.

Local Lesion.—In the subcutaneous tissues on the left side of the neck there was a very thin patch of fibrous tissue, about 1 in. in diameter, beset with discrete tubercles, the largest the size of a millet seed; the tubercles were grey, the smallest ones being almost transparent, the largest slightly opaque, though not definitely caseous, in the centre.

The Left Prescapular Gland was normal in size and nothing abnormal was seen on section.

Right Prescapular.—Normal.

Prepectoral and Cervical Glands.—Normal.

Thorax.

Parietal and Visceral Pleura.—Normal.

Lungs.—Normal except for two or three collapsed lobules.

Bronchial and Mediastinal Glands.—Normal.

Heart and Pericardium.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Spleen.—Weight 4½ ozs.—Normal.

Liver.—Normal.

Portal Glands.—Normal.

The Right Kidney showed on the surface of a lobule a group of about ten grey foci, the largest a little more than 1 mm. in diameter; on section these extended into the cortex in a radial manner; the larger ones were yellowish in the centre.

Left Kidney and Suprarenal Bodies.—Normal.

Tonsils, Pharynx and Intestines.—Normal.

Submaxillary, Pharyngeal, Gastric, Mesenteric and Ileo-Colic Glands.—Normal.

Testicles.—Normal.

Various Lymphatic Glands.

Cœliac, Lumbar, Renal, Iliac, Precrural, Pudic, Popliteal, Axillary and Parotid Glands.—Normal.

DOG 110. (Adult Retriever.)

Subcutaneous inoculation of culture derived from Guinea-pig 1707, inoculated with an emulsion made from the lung of Dog 50.

Date.—March 21st, 1906.

Dose.—Slightly less than 10 milligrammes.

Killed.—June 28th, 1906, 99 days after inoculation.

Clinical History.

The dog was inoculated subcutaneously in the right flank.

Fifteen days later there was a flat tumour measuring about 3 in. by $1\frac{3}{4}$ in., showing at one part a deep ulcer, $1\frac{1}{2}$ in. by $1\frac{1}{4}$ in., with slightly everted margins and floor formed by soft granulation tissue; there was a discharge of pus from the ulcer.

On April 12th, 1906, the ulcer was the size of a five-shilling piece and had abrupt clean cut margins. It then began to diminish in size; on May 10th, it was shallower and about $\frac{3}{4}$ in. in diameter and at the end of the experiment had almost disappeared.

On May 10th, a doughy swelling was noticed in the right axilla, the skin over which showed two or three small ulcers.

The general health of the animal was unaffected by the inoculation.

Tuberculin Test.—May 10th, 1906. 50 days after inoculation. Dose of tuberculin 5·0 cc. The temperature rose 1° C. twelve hours after injection.

Post-Mortem Examination.

Body in good condition.

Local Lesion.—The skin of the abdomen on the right side showed a superficial ulcer, about $\frac{1}{8}$ in. in diameter, with a pale granular floor; on the under surface of the skin around the ulcer there was a ring of white fibrous tissue from the margins of which strands of fibrous tissue radiated in all directions, giving a puckered appearance.

The Axillary and Inguinal Glands were normal in size and general appearance.

Thorax.

Lungs.—The lungs contained two grey slightly opaque tubercles the size of rape seeds; otherwise the lungs were normal.

Bronchial Glands.—Normal.

Heart and Pleura.—Normal.

Abdomen.

Spleen.—Normal.

Liver.—A grey point was seen in the liver.

Portal Gland.—Was normal.

Kidneys.—Each kidney showed in the cortex scattered grey tubercles, with opaque centres, the largest about 1·5 mm. in diameter (not apparently tuberculous).

Suprarenal Bodies.—Normal.

A Mesenteric Gland.—Contained a yellow speck.

Other Lymphatic Glands.—Normal.

Microscopical Examination (Smear Preparations).

Liver.—No tubercle bacilli.

Kidney.—No tubercle bacilli.

Mesenteric Gland.—No tubercle bacilli.

Lung.—Two tubercles.

DOG 108. (Adult Retriever.)

Fed with culture obtained from Guinea-pig 1707 (inoculated with an emulsion made from the lung of Dog 50); the culture was mixed with sterilized milk.

Date.—March 21st, 1906.

Dose.—10 milligrammes.

Killed.—June 27th, 1906, 98 days after.

Clinical History.

There was no sign of illness during the experiment.

Tuberculin Test.—May 10. 50 days after the experiment began. Positive. Rise of temperature, 1·3°C. Dose of tuberculin, 5·0cc.

Post-mortem Examination.

There was no tuberculosis of the mucous membrane of the alimentary tract or of the glands in connection with it.

Spleen.—Contained a pea-sized nodule which projected from the surface; on section the nodule consisted partly of spleen tissue and partly of soft greyish substance (? lymphoid); no tubercle bacilli were found in a smear.

Liver, Kidneys, and Suprarenal Bodies.—Were normal.

Thorax.

Lungs.—In the lungs there was one grey somewhat opaque submiliary tubercle; otherwise the lungs were quite normal.

Bronchial Glands.—Normal.

All other Organs and Glands.—Normal.

Microscopical Examination (Smear Preparations).

Lung.—No tubercle bacilli.

Spleen.—No tubercle bacilli.

DOG PASSAGE WITH VIRUS B XXII.

DOG 72.

Inoculated intraperitoneally on September 4th, 1905, with culture derived from the original material through Guinea-pig 1509.

Dose.—1·0 milligramme.

Killed.—February 7th, 1906, 156 days after inoculation.

Post-mortem Examination.

There were a few nodules and some fibrous tubercles in the lungs, a few yellow foci in the liver, and some doubtful tubercles in the kidneys.

A culture was isolated from one of the lung nodules and used to inoculate a series of rabbits and Dog 126.

All the rabbits died of general tuberculosis.

DOG 126.

Subcutaneous inoculation of culture, 2nd generation, 22 days old, derived from the lung of Dog 72.

Date.—May 9th, 1906.

Dose.—10 milligrammes.

Weight.—Initial weight 4,850 grammes. Final weight 6,000 grammes.

Killed.—August 16th, 1906, 99 days after inoculation.

Post-mortem Examination.

Local Lesion.—In the skin of the abdomen to the right of the middle line there was a small, flat, granulating ulcer.

In the skin over the inguinal glands there were three small apertures, two on the right and one on the left, which communicated with sinuses in the periglandular tissues.

The inguinal glands were not enlarged ; in the cortices there were a few fine, wavy, yellowish-white streaks.

Thorax.

Heart and Pleura.—Normal.

Lungs.—In the superficial part of the lung substance there were three translucent miliary tubercles.

Bronchial Glands.—Were small, one contained in the cortex a few whitish lines, a second contained a small irregular caseous focus.

Mediastinal Glands.—Normal.

Abdomen.

Peritoneum.—Normal.

Spleen.—Normal.

Kidneys.—Normal.

Liver.—Contained numerous translucent tubercles up to about 1 mm. in diameter ; occasionally a tubercle showed a minute central opacity

Portal Glands.—Normal.

Special Lymphatic Glands.

Mesenteric, Iliac, Lumbar, Submaxillary and Axillary Glands.—Normal

Microscopical Examination (Smear Preparations).

Liver.—A few scattered tubercle bacilli seen.

Lung.—No tubercle bacilli seen.

Bronchial Gland.—No tubercle bacilli seen.

PIG PASSAGE.

PIG 8.

Fed from October 1st, 1902, to December 31st, 1902 (92 days) with tuberculous milk of Cow 40.

Killed.—June 4th, 1903, 247 days after the experiment began.

Abstract from Post-Mortem Notes.

There were two or three submucous nodules in the small intestines, one of which was caseous; there was no ulceration; the mesenteric, pancreatic and ileo-colic glands were enlarged and caseous, and there was slight tuberculosis of the lungs, thoracic glands and liver.

Animals Inoculated.

An emulsion was prepared from the mesenteric glands and a pancreatic gland and inoculated into Pigs 10 and 12 and Calves 104 and 106.

PIG 10.

Subcutaneous.—Inoculation of an emulsion made from the mesenteric glands of Pig 8.

Dose of Emulsion.—10 cubic centimetres (no tubercle bacilli were found in the emulsion).

Date.—June 4, 1903.

Weight on June 9, 1 qr. 16 lbs.

Killed.—October 8, 1903. 126 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously on the inner side of the left thigh on June 4th, 1903.

A month after inoculation there was a subcutaneous thickening about $\frac{3}{4}$ inch in breadth with a cord-like prolongation into the groin.

On July 28th, 54 days after inoculation a small nodular mass was felt, around which was grouped a number of tubercles of various sizes; the skin was freely movable. Subsequently the mass increased a little in size and the tubercles in number.

The animal showed no signs of ill-health during the experiment.

Post-Mortem Examination.

Carcass.—In moderately good condition.

At the Seat of Inoculation.—On the inside of the left thigh, there was a small irregular tumour surrounded by a number (two or three dozen) of hard tubercles. The skin was freely movable over the tumour, but was adherent to a few of the tubercles. On section the tumour measured $1\frac{1}{2}$ in. by 1 in., and showed fibrous tissue enclosing softened caseous gritty material and a few discrete tubercles. The subcutaneous tubercles ranged in size from a pin's head to a small pea; each had a caseous gritty centre and a pinkish translucent periphery.

Similar tubercles infiltrated the underlying muscle.

Stretching from the under surface of the local tumour to the brim of the pelvis there was a thick cord; the distal end of the cord was one-third of an inch in diameter, and was composed of a pinkish translucent tissue, showing here and there a caseous focus, deposited around a thickened lymphatic vessel: the proximal end was narrower, and showed isolated tuberculous deposits.

Inguinal Glands.—The glands (five or six in number) in the left inguinal region, were fused together to form a mass weighing a little over an ounce, and measuring 3 in. by 1 in. by $1\frac{1}{2}$ in. On section they consisted of dense whitish caseous substance, slightly gritty from calcification; all the glands were easily shelled out from their capsules.

Iliac Glands.—The glands (six to eight in number) in the left iliac region were enlarged, and formed a mass 3 in. by 1 in. by $\frac{3}{4}$ in.: the larger ones contained numerous dense greyish white caseous masses, slightly gritty from calcification, the smaller ones were similarly, but not so extensively affected.

The Gland at the bifurcation of the aorta showed similar areas.

The Right Inguinal and the Right Iliac Glands.—Were not enlarged and showed no visible tubercles.

Abdomen.

In the Omentum, close to its attachment to the stomach were half-a-dozen tubercles ranging in size from a pin-head to a small pea; the small ones were grey and translucent; the large ones caseous and calcareous.

The Peritoneum covering the anterior surface of the stomach (in contact with the liver) showed a number of minute hard translucent granules. Elsewhere the peritoneum and omentum were normal.

Hepatic Glands.—Between the stomach and the anterior margin of the pancreas there was a mass of enlarged glands, from $\frac{1}{2}$ in. to $1\frac{1}{2}$ in. in length; this group of glands was continuous with the group in the hilum of the liver; all showed irregular caseous areas, as in the iliac glands, the smaller glands being less affected than the larger.

Gastric Glands.—Under the peritoneum on the posterior aspect of the stomach there were several enlarged glands, and a mass $2\frac{1}{4}$ in. by 1 in. tuberculous throughout.

Mesenteric Glands.—At the lower end of the mesentery, there were three enlarged glands, showing caseating areas, gritty from calcification. Other mesenteric glands and glands along the large intestine were normal.

Large and Small Intestine, Tongue, Tonsils, Pharynx, Larynx.—Normal.

Spleen.—Weighed 3 ozs. On the surface, and apparently growing in the capsule, were four small grey translucent tubercles; in the parenchyma there were between twenty and thirty tubercles, ranging in size from a pin-head to a small pea; on section they were all caseous and gritty. Some of these tubercles caused projections from the surface and one little group (an eighth of an inch in diameter) was surmounted by a vascular tuft.

Splenic Lymphatic Glands.—Near the centre of the hilum there was a gland the size of a filbert, showing gritty caseating areas.

Liver.—Weighed 1lb. 12oz; beneath the capsule, and moderately evenly distributed throughout the parenchyma, were numerous tubercles; the larger ones, the size of a small pea, had on section a lobular outline, appearing as if made up of several tubercles; they were for the most part greyish, firm and homogeneous throughout; a small proportion, however, showed yellow calcareous centres.

Hepatic Glands.—(*See* above).

Kidneys.—Normal.

Suprarenal Bodies.—Normal.

Thorax.

Pleura.—A little to the left of the middle of the tendon of the diaphragm there was a hard grey nodule a quarter of an inch in diameter.

On the pleural surface of the diaphragm, chiefly in the centre and along the margins of the tendon, there were some pale villous-like growths; at the base of some of these minute hard granules could be felt. Of those along the margin of the tendon a few had towards their free ends small tubercles, two of which were reddish, caseous and calcareous.

Similar growths were seen on the pleura covering the last ribs; these growths were very long and slender; one on each side contained at the free extremity a flattened chalky-white body, the size of a wheat grain, almost entirely detached; one such body was found free in the right pleural cavity. On section they were dense, dry and caseous.

Near the apex of the somatic pleura on the left side there was a small grey tubercle.

Lungs.—Scattered evenly throughout the lungs were numbers of hard grey tubercles; they varied in size from a mustard seed to a small pea; all were caseous and softened in the centre; nearly all were appreciably calcified.

The lung tissue between the tubercles appeared to be normal.

Mediastinal and Bronchial Glands.—The mediastinal and bronchial glands were very large. One dorsal mediastinal gland measured 2 in. by $1\frac{1}{8}$ in., two behind this were an inch and half an inch in length respectively.

Bronchial Glands.—Each bronchial gland measured 1½ in. by 1 in. by ½ in., and showed areas similar to those described in the iliac glands.

Two glands along the œsophagus, one above the treachea, and a group at the opening of the thorax (above the manubrium sterni) were similarly affected.

Heart and Pericardium.—Normal.

Thymus and Thyroid.—Normal.

Brain and Meninges.—Normal.

Ovaries.—Were cystic; in the tissue between the cysts there were several small greyish points.

Uterus and Fallopian Tubes, Pancreas.—Normal.

Various Lymphatic Glands.

Submaxillary Glands.—The left submaxillary gland was normal; one on the right side was enlarged and tuberculous.

Left Parotid.—Showed a caseous nodule.

Right Parotid, Right and Left Prescapular, Right and Left Cervical, Right and Left Popliteal, Right and Left Lumbar, Right and Left Posterior Pharyngeal.—Normal.

Microscopical Examination (*Smear Preparations*).

Emulsion of Inguinal Glands.—Twenty-seven tubercle bacilli.

Liver.—No tubercle bacilli.

Lung.—No tubercle bacilli.

Pleura (*free body*).—No tubercle bacilli.

Spleen.—No tubercle bacilli.

Omentum.—One tubercle bacillus.

Gastric Gland.—No tubercle bacilli.

Mediastinal Gland.—No tubercle bacilli.

Animals Inoculated.

An emulsion was made from the inguinal glands, and inoculated into :—

G. P. 629.	(S.)	Died 69 days.	General tuberculosis.
G. P. 630.	(S.)	Died 67 days.	General tuberculosis.
G. P. 631.	(I.)	Died 19 days.	Tuberculosis.
G. P. 632.	(I.)	Died 29 days.	Tuberculosis.

An emulsion was made from one ovary, and inoculated into :—

G. P. 633. Killed 70 days. No tuberculosis.

PIG 12.

Subcutaneous Inoculation of an emulsion made from the mesenteric glands of Pig 8.

Dose of Emulsion.—10 cubic centimetres. (No tubercle bacilli were found in the emulsion.)

Date.—June 4th, 1903.

Weight.—June 9th, 1903, 1 qr. 10 lbs.

Killed.—October 2nd, 1903, 120 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously on the inside of the left thigh on June 4th, 1903.

A month after inoculation there was a tumour measuring 1½ inches by ¾ inch by about ½ inch, the skin over which was adherent and reddened.

The tumour slowly increased in size, and on August 6th, 1903, 63 days after inoculation, measured 2½ inches by 1½ inch. The tumour then diminished a little and a small ulcer appeared in the skin.

On July 7th, 33 days after inoculation, the glands (three in number) in the groin were enlarged, but separate; they increased in size and became adherent, forming a mass about the size of a small hen's egg.

There was no disturbance of the general health of the animal during the experiment.

Post-Mortem Examination.

The carcass was in good condition; fat everywhere moderately abundant.

At the Seat of Inoculation.—On the inside of the left thigh there was a slightly elevated firm tumour 1¾ in. by 1 in. by ½ in., the skin over it was adherent, dull red, and thinned, and showed at the centre a small ulcer.

On section the tumour consisted of fibrous tissue interspersed with dense chalky white caseous areas. The caseous deposits were more numerous at the periphery of the mass than in the centre. All were gritty from calcification.

In the subcutaneous tissue around the tumour there were perhaps a dozen caseous and calcareous tubercles about the size of wheat grains.

Inguinal Glands.—The glands (five or six in number) in the left inguinal region were enlarged and glued together to form a nodular mass 3 in. by 1 in., weighing one ounce. On section the glands were almost entirely caseous; they were surrounded by thin fibrous capsules, from which they were easily shelled out; the caseous substance was white and dense and gritty from calcification.

One gland in the right inguinal region was slightly enlarged, and showed on section dense greyish-white caseous masses, slightly gritty from calcification; a second gland, not apparently enlarged, contained a small caseous focus; a third gland in this series was normal.

A gland at the bifurcation of the aorta was enlarged and infiltrated throughout with caseous gritty areas.

Iliac Glands.—On the left side two were similarly, but not so extensively affected; one right iliac gland, not enlarged, showed a white gritty caseous focus.

Abdomen.

Omentum and Peritoneum.—Normal.

Spleen.—Weighed 2 ounces. Distributed evenly throughout the parenchyma was a number (thirty to forty) of grey tubercles, the size of a large shot; some formed hemispherical projections beneath the capsule; almost all had caseous and calcareous centres.

Liver.—Weighed 1 lb. 12 ozs. On the surface, and uniformly distributed throughout the parenchyma, were numerous greyish tubercles, smaller than those in the spleen; a few only of these showed central caseation. The glands in the hilum were enlarged, and showed similar areas to those described in other glands.

Kidneys.—The left kidney weighed three ounces, and showed beneath the capsule in the cortex two grey translucent tubercles, the size of mustard seeds.

The right kidney weighed three ounces; the cortex contained one tubercle as above.

Suprarenal Bodies.—Normal.

Ovaries.—The size of French beans, were cystic, and showed in the little triangular areas between contiguous cysts a number of grey specks.

Uterus, Fallopian Tubes.—Normal.

Tongue, Pharynx, Larynx, Tonsils.—Normal.

The Glands at the angle of the jaw, parotid and submaxillary, three in number on each side, were firm and enlarged (the largest about the size of a thrush's egg); on section they showed greyish-white translucent areas forming a network throughout the gland tissue; these areas were not softened nor appreciably calcareous, but showed opaque whitish streaks in their centres.

Posterior Pharyngeal Glands, Right Cervical, and Left Prescapular Glands.—Were similarly affected, but not to the same extent.

Left Cervical, Right Prescapular.—Were apparently normal.

The Glands below the trachea, at the opening of the thorax, were enlarged and contained cascating areas.

There was a mass (about the size of a tangerine orange) of enlarged glands between the stomach and the anterior border of the pancreas, which on section were extensively caseated.

The Mesenteric Glands and glands along the whole length of the large intestine, were, without exception, slightly enlarged and showed similar areas of caseation; the areas in the mesenteric glands were more softened than those in other glands.

Lumbar Glands.—Showed a few caseous foci.

Large and Small Intestine.—Normal.

Thorax.

Lungs.—Uniformly scattered throughout normal lung tissue were numerous grey translucent tubercles, of the size of a pin's head; a number were grey and homogeneous throughout, but many showed minute yellow calcareous centres.

Bronchial and Mediastinal Glands.—Were enlarged and showed caseating areas and foci gritty from calcification. The ventral mediastinal glands were similarly affected.

Diaphragm.—The abdominal surface of the diaphragam carried on the central tendon from six to eight prominent rounded hard translucent tubercles, which varied in size from a mustard seed to a wheat grain.

On the pleural surface, there were about half a dozen similar tubercles.

Costal Pleura, Heart, Pericardium.—Normal.

Microscopical Examination (Smear Preparations).

The Emulsion.—Showed tubercle bacilli.
Spleen.—Showed no tubercle bacilli.
Lung.—Showed one tubercle bacillus.
Kidney.—Showed no tubercle bacilli.
Submaxillary Gland.—Showed one tubercle bacillus.
Left Inguinal Gland.—Showed no tubercle bacilli.
Emulsion of Mediastinal Gland, (used to inoculate G.P's.)—Showed one tubercle bacillus.

Paraffin Sections.

Sections through one of the grey tubercles in the kidney showed small cell groups, the central parts of which were necrosed; no giant cells or tubercle bacilli were seen (in four sections).

Sections through one of the ovaries showed clusters of small cells in the position of the grey foci; there was nothing in the arrangement of these to indicate a tuberculous origin or nature (*i.e.*, no giant cells, degeneration or tubercle bacilli).

Animals Inoculated.

An emulsion was made from the inguinal glands, part of the spleen and two hepatic lymphatic glands; the emulsion contained 900 tubercle bacilli per c.c.

Of this Emulsion,

Calf 124, Calf 130, Pig 14, Pig 16,	Each received 10 cc. subcutaneously.
G.P. 602, G.P. 603	1 c.c. subcutaneously.
G.P. 605, G.P. 604,	1 c.c. intraperitoneally.

Emulsion of Mediastinal Gland.

G.P. 606. (Subcutaneously.)

Of the guinea-pigs inoculated with the first emulsion, one died 18 days later and showed only slight tuberculosis; the rest died of general tuberculosis in periods of 60 to 71 days.

Guinea-pig 606 was killed 76 days after inoculation and showed general tuberculosis.

CALF 104.

Subcutaneous.—Inoculation of an emulsion made from the mesenteric glands of Pig 8.

Dose.—10 c.c. (No tubercle bacilli were found).

Date.—June 4th, 1903.

Weight.—June 9th, 1903. 1 cwt.

Killed.—September 11th, 1903. 99 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on June 4th, 1903.

Three weeks after inoculation a small thickening was felt in front of the inferior extremity of the prescapular gland. A week later, this had increased to form a small nodular mass about 1 inch in diameter. Later discrete hard nodules were felt around the mass and a few appeared in the tissues posterior to the gland.

The prescapular gland, three weeks after inoculation, measured 2½ inches by 1½ inch, and at the end of five weeks attained its maximum, 3½ inches by 2½ inches.

The general condition of the calf remained good throughout the experiment.

The temperature rose on the 22nd day to 40·1° C.; it then gradually fell to 39·3° C. on the 25th day, and during the rest of the experiment was practically normal.

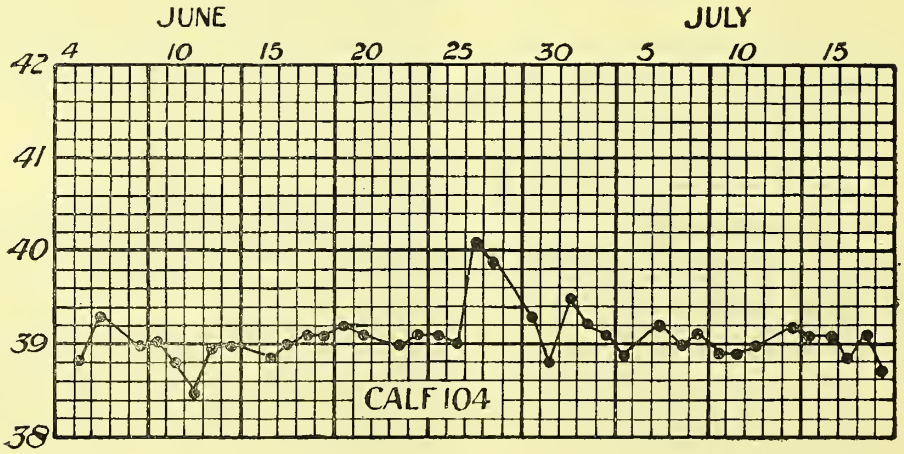
The tuberculin test on September 4th, 1905, 93 days after inoculation, was positive, the temperature rising 2·1° C.

Weights.

During the first seven weeks there was a gain in weight of 52 lbs.; then for a fortnight the weight was stationary; subsequently there was a steady increase in weight.

June 9, 1903 - -	1 cwt.	0 qr.	0 lb.
July 28, 1903 - -	1 cwt.	1 qr.	24 lbs.
August 11, 1903 -	1 cwt.	1 qr.	24 lbs.
September 8, 1903 -	1 cwt.	2 qrs.	10 lbs.
Gain in weight -		2 qrs.	10 lbs.

Temperature.



Post-Mortem Examination.

Carcass.—In moderately good condition.

Local Lesion.—On reflecting the skin from the left side of the neck, numerous yellow hard tubercles were seen in the subcutaneous tissue.

The tubercles lay for the most part anterior to the gland, but there was a small number situated postero-inferiorly. They varied in size from a small shot to a small pea. At one point anterior to the lower end of the gland they formed a nodular mass; elsewhere they were discrete. Some were closely adherent to the skin, others to the aponeurosis of the muscle. On section all had calcareous centres surrounded by pinkish translucent tissue. In the areolar tissue between the superficial and deep muscles, and 2 in. in front of the gland, was a small collection (twelve to fifteen) of similar calcareous tubercles; three others were situated nearer the gland.

The total number of tubercles was not more than 100.

Left Prescapular Gland.—Weighed three ounces, and measured 3 in. × $1\frac{3}{4}$ in. × $1\frac{1}{4}$ in. The gland tissue was firm, and the cortex was extensively infiltrated with yellow calcareous particles; near the hilum at the upper end there was an area of caseo-calcareous tissue; the calcareous particles were not so numerous at the lower end of the gland.

Lungs.—Irregularly distributed over the surface, but chiefly on the middle and posterior lobes, were numbers of small tubercles; some were seen just beneath the pleura, others projected. On section they varied in size from a small shot to a large pea and had calcareous centres, and were surrounded by an irregular zone of congestion. On the upper surface of the right lobe there were in addition two pinkish-red flattened tubercles with overhanging edges: the small one ($\frac{1}{8}$ in. in diameter) had a fringe around its margin, the large one ($\frac{3}{8}$ in. in diameter) calcareous grains at its base. On each dorsal surface of the posterior lobes there were three similar mushroom like growths, $\frac{3}{10}$ in. in diameter, surrounded by fringes.

On section of the lung, tubercles, the size of a No. 4 shot, were moderately numerous throughout the parenchyma; all were calcareous in the centre. The lung tissue apart from the tubercles was quite normal.

Around the margins of the lungs were delicate fibrinous fringes in which were felt small hard tubercles, grey in colour; delicate fringes were seen on the surfaces of the posterior and middle lobes, arranged along the lines of the interlobular septa.

Pleura.—On the anterior margins of the ribs, chiefly on the right side, were rows of firm pinkish vegetations, in which were, here and there, small hard grey nodules: similar vegetations were seen on the pleura covering the aorta, and a small number occurred at the junction of the muscle and tendon of the diaphragm, and on its central pillars; some of the vegetations on the diaphragm contained yellow caseous tubercles.

Mediastinal Glands.—The dorsal mediastinal glands, four in number, weighed four ounces: they were enlarged, and on section the gland tissue was very congested; in the long posterior gland (4 in. in length) there were numerous calcareous nodules affecting about half the cortex; the remaining three glands were calcareous throughout.

Three small glands along the thoracic trachea contained calcareous nodules.

Bronchial Glands.—Both were enlarged, the left weighing three ounces, and practically completely calcareous.

Heart.—On the left border of the left ventricle there was a prominent white firm oval nodule, the size of a filbert, homogeneous throughout, except for a small lacuna in the centre containing a greenish fluid. Microscopically the tumour was a fibroma.

Intestines.—Normal.

Mesenteric Glands.—Two glands, one at the upper and one at the lower end of the mesentery, each contained a small calcareous nodule.

Colic and Gastric Glands.—Normal.

Spleen.—Weighed nine ounces; on both surfaces there were several small red vascular tufts loosely connected to the capsule. On section there were in all about six shotty tubercles with calcareous centres.

Liver.—On the convex surface of the liver there were from fifteen to twenty small greyish-white tubercles, some just visible through the capsule, others projecting above the surface; also three yellowish white firm growths, from $\frac{1}{8}$ to a $\frac{1}{4}$ in. in diameter, with thin overhanging margins and a slender attachment to the capsule. On the concave surface there were two hemispherical tubercles resembling small pearls.

On section, tubercles the size of a pin's head, calcareous in the centre, were sparsely distributed throughout the liver tissue.

Three Hepatic Lymphatic Glands.—Contained numerous calcareous particles in the cortex, the largest gland being the most affected.

Kidneys.—Each weighed six ounces. Both were normal on the surface and section.

Suprarenals.—Normal.

Tongue, Pharynx, and Tonsils.—Normal

Mammary Gland, Uterus.—Normal.

Various Lymphatic Glands: Left Prepectoral, Right Prepectoral, Right Prescapular, Axillary, Cervical, Submaxillary, Retro-pharyngeal, Lumbar, Iliac, Precrural, Popliteal, Gluteal, Ischial, Supramammary.—Normal.

Microscopical Examination (Smear Preparations).

Local Tumour.—No tubercle bacilli.

Left Prescapular Gland (Emulsion).—Tubercle bacilli.

Mesenteric Gland.—Tubercle bacilli.

Liver.—Tubercle bacilli.

Lung.—A few tubercle bacilli.

Spleen (Emulsion).—One tubercle bacillus.

Nodule on Diaphragm.—Two or three tubercle bacilli.

Animals Inoculated :—

With Emulsion of Left Prescapular Gland:

G. P. 562.—(S.) Died in 29 days of general tuberculosis.
G. P. 563.—(S.) Died in 38 days of general tuberculosis.
G. P. 564.—(I.) Died in 20 days of general tuberculosis.
G. P. 565.—(I.) Died in 29 days of general tuberculosis.

With Emulsion of Spleen (two tubercles):

G. P. 566.—(S.) Died in 39 days of general tuberculosis.

CALF 106.

Subcutaneous Inoculation of an emulsion made from the mesenteric glands of Pig 8.

Dose.—10 cubic centimetres. (No tubercle bacilli were found in the emulsion).

Date.—June 4th, 1903.

Weight.—June 9th, 1903. 1 cwt., 0 qr., 21 lbs.

Killed.—September 12th, 1903. 100 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on June 4, 1903.

Three weeks after inoculation a small irregular mass was felt at the lower extremity of the left prescapular gland. This increased in size a little and became very hard and nodular, and discrete nodules were felt in the subcutaneous tissues over the gland and along its posterior margin.

The prescapular gland, three weeks after inoculation, measured $2\frac{1}{2}$ in. by $1\frac{1}{2}$ in.; it slowly enlarged and at the end of seven weeks measured nearly 4 in. in length; it then underwent a slight diminution in size.

The general health of the calf remained good throughout the experiment.

The temperature rose sharply to 40·4°C. on the 22nd day and then gradually fell to the normal on the 25th ; during the rest of the experiment the temperature was normal.

Tuberculin Tests.—September 4, 1903, 92 days after inoculation. Positive. Rise of temperature 2·3°C

September 11, 1903, 99 days after inoculation. Positive. Rise of temperature 1·5°C.

Weights.

The weight steadily increased.

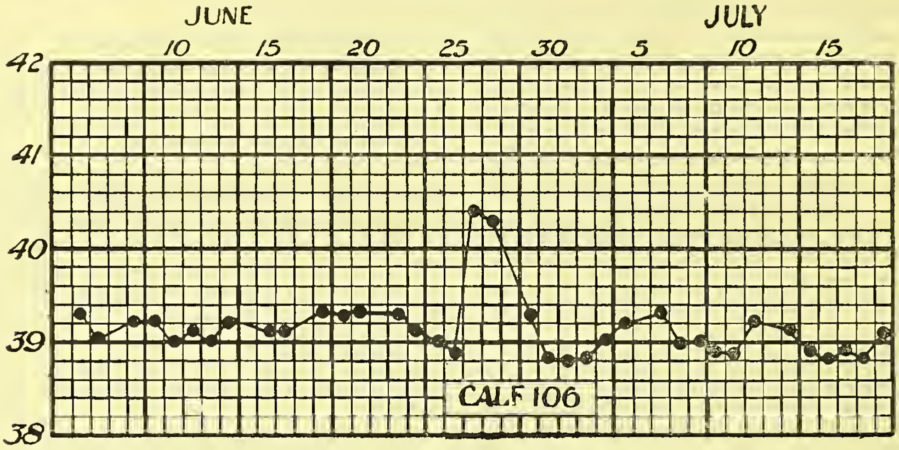
June 9, 1903.—1 cwt. 0 qr. 21 lbs.

September 8, 1903.—2 cwt. 0 qr. 4 lbs.

Gain in weight, 3 qrs. 11 lbs.

Rate of gain per week, 7·3 lbs.

Temperature.



Post-Mortem Examination.

Carcass in very good condition.

Local Lesion.—In the subcutaneous tissue immediately over that part of the sterno-mastoid muscle which covered the left prescapular gland were five yellow tubercles, varying in size from a small shot to a large pea ; on section they had calcareous centres surrounded by pinkish translucent tissue.

In the loose tissue around the lower end of the left prescapular gland was a collection of similar yellow tubercles, numbering about 100. The tubercles were grouped chiefly around the inferior extremity of the gland, but they also extended half way up the posterior margin and slightly along the anterior ; in the latter situations they were discrete and varied in size as above ; in the former they formed nodular masses ; all on section were calcareous in the centre.

Left Prescapular Gland.—Weighed 3 ounces and measured 3 in. by 1¾ in. On section the upper two-thirds of the cortex of the gland contained large caseo-calcareous masses ; the lower third discrete calcareous particles.

Right Prescapular Gland.—Weighed a little over 1 ounce and measured 2½ in. by 1¼ in. ; the tissue was œdematous, but there were no hæmorrhages.

At the seat of inoculation of the tuberculin on the right side there was a fairly well defined œdematous swelling, 5 in. by 3½ in. On section through the swelling the œdema was seen to infiltrate the underlying muscle, and to extend into the deep areolar tissue.

Thorax.

Lungs.—Normal on surface and on section.

Mediastinal Glands.—In the cortex of the long dorsal mediastinal gland there was a small yellow calcareous tubercle.

Other mediastinal and the bronchial glands were normal.

Pleura and diaphragm, normal.

Abdomen.

Omentum and Peritoneum.—Normal.

Spleen (9¾ ozs.).—Showed on both surfaces a few very small vascular tufts loosely attached to the capsule : on section normal.

Liver.—Normal.

Hepatic Lymphatic Glands.—Normal.

Kidneys and Suprarenal Bodies.—Normal.

Tongue, Tonsils, Pharynx } Normal.
Intestines, Mammary Glands }

Other Lymphatic Glands.

Submaxillary, Posterior Pharyngeal, Cervical, Right and Left Prepectoral, Right and Left Axillary, Precrural, Popliteal, Gluteal, Ischiatic, Iliac, Lumbar, Gastric, Colic and Mesenteric Glands.—Normal.

Microscopical Examination (Smear Preparations).

Left Prescapular Gland.—Numerous T.B.

Tubercle from Local Lesion.—A few T.B.

Nodule from Mediastinal Gland.—No T.B.

Animals Inoculated.

With Emulsion from Left Prescapular Gland :—

G.P. 567. (S.) Died in 69 days of general tuberculosis.
G.P. 568. (S.) Died in 41 days of general tuberculosis.
G.P. 569. (I.) Died in 28 days of general tuberculosis.
G.P. 570. (I.) Died in 29 days of general tuberculosis.

With Emulsion from Spleen :—

G.P. 571. (S.) Died in 69 days of general tuberculosis.

PIG 14.

Subcutaneous Inoculation of an emulsion made from the inguinal glands, two hepatic lymphatic glands and part of the spleen of Pig 12.

Dose of Emulsion.—10 c.c., containing about 9,000 tubercle bacilli.

Date.—October 2nd, 1903.

Weight.—October 6th, 1903. 1 cwt., 0 qr., 25 lbs.

Killed.—January 25th, 1904. 115 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously on the inside of the left thigh on October 2, 1903.

A fortnight after inoculation there was a tumour the size of a filbert, the skin over which was adherent ; the inguinal glands were a little enlarged. Forty days after inoculation the tumour measured 3 in. by 1½ in. by about 1 in. ; the skin was thoroughly adherent, dull red and scaly ; in the groin there was a mass of enlarged glands.

On December 9th the centre of the tumour was felt to be softened and the skin in the centre was depressed, thinned, and showed some adherent scabs. Thickened lymphatic vessels stretched between the tumour and the mass of enlarged glands in the groin. Subsequently the tumour ulcerated and diminished somewhat in size.

No signs of ill-health were observed during the experiment; the appetite remained good, and there was a gain in weight; the pig, however, was not so fat, and did not grow at the same rate, as two control pigs belonging to the same litter.

The temperature rose on the 7th day to 39·5°C., and fell to the normal on the next day; it rose again on the 18th day and was high for three days (maximum 39·9°C.); subsequently, except for two slight rises, one to 39·3°C., the temperature was normal.

Weights.

During the first four weeks there was a steady gain in weight, amounting altogether to 31 lbs.: during the remainder of the experiment the weight fluctuated between 1 cwt. 1 qr. 14 lbs. and 1 cwt. 2 qrs. 15 lbs.

October 6th, 1903	- 1 cwt.	0 qrs.	25 lbs.
November 3rd, 1903	- 1 cwt.	2 qrs.	0 lbs.
December 1st, 1903	- 1 cwt.	1 qr.	14 lbs.
December 22nd, 1903	- 1 cwt.	2 qrs.	15 lbs.
January 19th, 1904	- 1 cwt.	2 qrs.	9 lbs.
Gain in weight-	-	1 qr.	12 lbs.

Post-Mortem Examination.

Condition.—Moderate.

At the Seat of Inoculation.—On the inside of the left thigh, there was a ridge 3 inches long, ¾ inch broad, and about ¾ inch high. In the centre of this ridge there was a narrow shallow ulcer, with puckered margins, a little over 1 inch long; the ulcer at the anterior end discharged thick yellow pus, but elsewhere was cicatrised.

When cut into the ridge was found to be composed of fibroid tissue containing, principally in the deeper parts, scattered caseous and softened tubercles.

In the subcutaneous tissue adherent to this ridge there were a few discrete yellow tubercles, appreciably gritty.

Between the local lesion and the inguinal glands there was a slightly thickened lymphatic vessel, which showed here and there along its course caseous nodules; the lumen of the vessel was patent.

Inguinal Glands.—The superficial inguinal glands were enlarged, and fused together to form a mass 3 inches by $1\frac{1}{2}$ inches by 3 inches, weighing 2 ounces. On section all the glands were caseous throughout, with the exception of one or two small glands at the margin of the mass, which contained discrete yellow foci; the caseous areas were appreciably gritty.

The superficial inguinal glands on the right side were normal in size and appearance.

Iliac Glands.—The iliac glands on the left side (five in number) were enlarged and indurated; the posterior one of the series, the size of a chestnut, was entirely caseous; the four glands anterior to this, and a quarter the size, were beset with caseous foci.

The iliac glands on the right side were slightly indurated but were otherwise normal.

Lumbar Glands.—Normal.

Abdomen.

Omentum, Peritoneum.—Normal.

Spleen.—Weighed 4 ounces, and showed no sign of tubercle either on the surface or on section.

Liver.—Showed on the surface beneath the capsule half-a-dozen minute whitish foci; in the depth of the liver substance there was a minute yellow focus with a gritty centre.

Kidneys.—The right kidney contained in the cortex, near the surface, a grey translucent point, just visible to the naked eye. Left kidney normal.

Suprarenals.—Both normal.

Gastric Glands.—On the posterior wall of the stomach, near the lesser curvature, and beneath the peritoneum, there was a group of glands, one of which contained a caseous, slightly calcareous nodule. One or two glands similarly placed, but on the anterior wall, were normal.

Portal Glands.—In the interval between the pancreas and the hilum of the liver there were four or five enlarged and indurated glands, which on section showed numerous caseous gritty foci.

Mesenteric Glands, Colic Glands.—Normal in size, showed no visible tubercles.

Small Intestine, Large Intestine.—Normal.

Thorax.

Lungs.—Contained a number (about thirty were counted in each lung) of caseous tubercles, ranging in size from a mustard seed to a large shot; the tubercles were softened in the centre, and the larger ones had distinct fibrous capsules.

Bronchial Glands.—On the left side were considerably enlarged, and closely beset with caseous tubercles.

The glands on the right side were only slightly enlarged, but contained similar caseous foci.

The glands in the dorsal mediastinum appeared normal.

Pleura (Parietal and Visceral), Heart, Pericardium.—Normal.

Larynx and Trachea.—Normal.

Right and Left Cervical (Superficial and Deep), Right and Left Prescapular Glands.—Normal.

Right and Left Prepectoral, Right and Left Precrural Glands.—Normal.

Post-Pharyngeal Glands, Submaxillary Glands.—Normal.

Testicles.—Both testicles, and the peritoneum covering them, were normal.

Over the site of the inoculation of the tuberculin (inside of right thigh) there was an area of desquamation of the superficial epithelium.

Microscopical Examination (Smear Preparations).

Left Inguinal Glands (Emulsion).—Two tubercle bacilli.

Liver.—No tubercle bacilli.

Lung.—No tubercle bacilli.

Guinea-Pigs Inoculated.

Guinea-pig 826 was inoculated subcutaneously with an emulsion from the spleen; it was killed after 97 days and found to be free from tuberculosis.

Guinea-pig 827, inoculated subcutaneously with an emulsion made from the liver, died in 95 days of general tuberculosis.

Guinea-pig 829, inoculated intraperitoneally with an emulsion from an inguinal gland, died in 34 days of general tuberculosis.

PIG 16.

Subcutaneous Inoculation of an emulsion made from the left inguinal glands, two hepatic glands and part of the spleen of Pig 12.

Dose of Emulsion.—10 c.c., containing about 9,000 tubercle bacilli.

Date.—October 2nd, 1903.

Killed.—January 16th, 1904. 105 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously on the inside of the left thigh on October 2nd, 1903.

A fortnight after inoculation there was a tumour adherent to the skin, about the size of a filbert; the glands in the groin were a little enlarged.

Sixty-eight days after inoculation the tumour measured 5 in. by 2 in., and thickened lymphatic vessels were felt between it and the mass of enlarged glands in the groin; the skin over the centre of the tumour showed a few dry scabs.

The tumour then diminished a little in size and the skin in the centre became depressed, thinned and finally ulcerated.

The general condition of the animal remained good throughout the experiment.

The temperature rose on the seventh day to 39·6° C., and during the first month there was slight irregular pyrexia (maximum 39·4°C.); subsequently, except for one or two slight rises, the temperature was normal.

Weights.

October 6th, 1903 -	- 1 cwt. 0 qrs. 7 lbs.
January 12th, 1904	- 1 cwt. 1 qrs. 24 lbs.
Gain in weight -	- 1 qr 17 lbs.

Post-Mortem Examination.

Condition.—Moderate.

Local Lesion.—Extending across the surface of the left thigh at the seat of inoculation there was a ridge about 4½ in. in length, and about ¾ in. in width at the widest part. At the base of the ridge, on its lower side, there was a narrow ulcer over an inch in length.

On dissection this ridge was found to be mainly composed of an aggregation of tubercles, mostly with yellow caseous centres. The skin was partially adherent to the mass.

Leading forwards from this lesion to the superficial inguinal glands on the same side there was a number of lymphatic vessels of the diameter of a goose quill. On section these were found to have no distinct lumen, and were almost entirely caseous.

Inguinal Glands.—The left superficial group of inguinal glands formed a single mass weighing 3½ ozs., and measuring 3½ in. by 2 in. by 1¼ in. On section the glands were necrotic and caseous throughout. The caseous tissue was firm, and was readily shelled out from the surrounding gland capsules.

On the right side the inguinal glands were of about normal size, but slightly indurated, and showed one or two points of caseation.

Iliac Glands.—On the left side the iliac glands were enlarged to three or four times the normal size, and were closely beset with caseous tubercles.

Both iliac and superficial inguinal glands were just perceptibly calcified.

Right iliac glands normal in size and appearance.

Lumbar Glands.—Normal in size and appearance.

Abdomen.

Omentum.—Normal.

Peritoneum.—The peritoneum covering the diaphragm on the right side showed two clusters of tubercles; there were also two or three tubercles on the peritoneum covering the right crus of the diaphragm (the tubercles on the tendon of the diaphragm caused subpleural as well as subperitoneal elevations); elsewhere the peritoneum, both parietal and visceral, appeared normal.

Spleen.—The spleen was of normal size, but in its substance there were about a dozen tubercles, the largest being about the size of a pea; these were caseous centrally, and had distinct fibrous capsules.

The Liver contained numerous tubercles underneath the peritoneum, also throughout the substance; these were about the size of pin-heads.

Kidneys and Suprarenal Bodies.—Normal.

Gastric Glands.—The gastric group of glands was enlarged, and almost completely destroyed by caseation.

Stomach.—Normal.

Intestines.—Several Peyer's patches showed suspicious opacity of their lymphoid tissues (a smear preparation was made from one of the patches, but no tubercle bacilli were found). Otherwise normal.

Large Intestine.—Normal.

Mesenteric Lymphatic Glands, Colic Lymphatic Glands were normal in size, and showed no visible lesions.

Tongue, Throat, Tonsils, Post-Pharyngeal Lymphatic Glands, Submaxillary Glands.—Normal.

Thorax.

Lungs.—Both lungs contained numerous tubercles (some hundreds), mostly about the size of barley grains or smaller; these had distinctly caseous centres.

The Bronchial Glands on both sides were enlarged, and closely beset with caseous tubercles.

Pleura.—Several yellow tubercles about the size of barley grains were present in the mediastinal pleura behind the heart, and also in the special fold of pleura surrounding the vena cava.

On each side of the chest wall, under the pleura covering an intercostal space, there was a single tubercle.

Pericardium, Heart Muscle and Valves.—Normal.

Thymus.—Normal.

Cervical Glands, Prescapular and Prepectoral.—Normal on both sides.

Right Popliteal.—Normal.

Testicles.—Under the peritoneum covering the left spermatic cord and epididymis there were about half a dozen firm tubercles, the largest about the size of a barley grain. The testicle itself, and the substance of the epididymis, appeared normal.

Bladder.—Normal.

Microscopical Examination (Smear Preparations).

Emulsion of Inguinal Glands.—Tubercle bacilli (not numerous).

Liver.—No tubercle bacilli.

Spleen.—Tubercle bacilli (scanty).

Intestine (Peyer's Patch) }
Lung. } No tubercle bacilli.

Animals Inoculated.

With Emulsions.

Four guinea-pigs were inoculated, two subcutaneously, and two intraperitoneally, with an emulsion made from the left inguinal glands; all died of general tuberculosis in periods varying from 24 to 56 days.

With Culture.

A culture was obtained from an inguinal gland and inoculated into two rabbits (*see* below), two pigs, and a calf.

Rabbits.

No. of Rabbit.	Initial Weight in grammes.	Final Weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
159	1,700	1,200	1·0 mg.	Intravenous	Died, 21 days	General tuberculosis.
158	1,000	1,070	1·0 mg.	Intraperitoneal	Died, 62 days	General tuberculosis; the inoculation was partly subcutaneous.

CALF 124.

Subcutaneous Inoculation of an emulsion made from the inguinal and hepatic lymphatic glands and the spleen of Pig 12.

Dose.—10 cubic centimetres, containing approximately 9,000 tubercle bacilli.

Date.—October 2, 1903.

Weight.—1 cwt.

Died.—December 12, 1903, 71 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on October 2nd, 1903.

A fortnight after inoculation there was a small local thickening. At the end of the third week there was a tumour measuring 3½ in. by 2½ in. by about 1·in.; this slowly increased in size, and at the end of the 8th week measured 5 in. by 3 in.

The left prescapular gland at the end of a fortnight was slightly enlarged, and on November 20th, seven weeks after inoculation, measured nearly 5 in. in length.

The calf was first noticed to be ill about seven weeks after inoculation; the coat was staring and there was some loss of appetite. On November 28th, 57 days after inoculation there was slight diarrhœa, and on November 30th, the respirations were visibly increased. These symptoms increased in severity; diarrhœa became more marked: the respirations were rapid and laboured, expiration ending audibly; the appetite was poor and there were progressive emaciation and weakness.

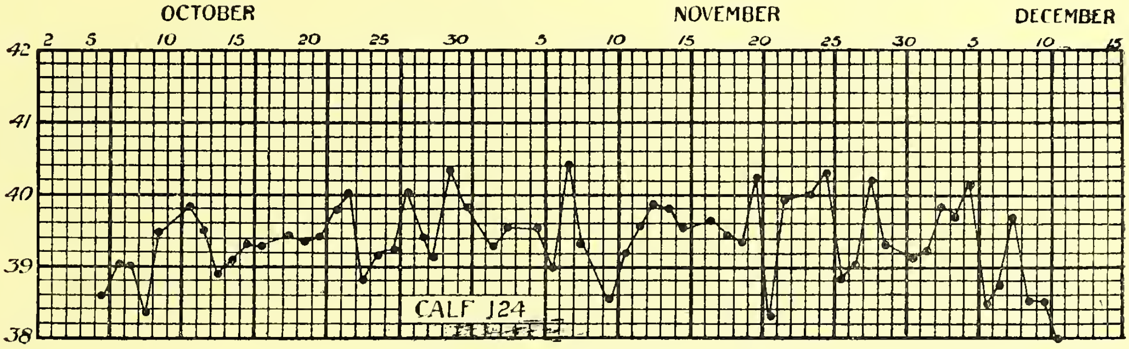
On the day before death the calf was very weak and emaciated and almost unable to stand; the head was outstretched, and respiration was very laboured.

Weights.

During the first month there was a gain in weight of 15lbs., then there was a gradual decline.

October 6th, 1903 -	1 cwt.	0 qr.	0 lb.
November 3rd, 1903 -	1 cwt.	0 qr.	15 lbs.
December 8th, 1903 -	0 cwt.	3 qrs.	22 lbs.
Loss in weight - - - -			6 lbs.

Temperature.



Post-Mortem Examination.

Carcass.—Very emaciated.

At the Seat of Inoculation.—On the left side of the neck, in the subcutaneous tissues, there was a rounded prominent tumour 4 in. by 2 in. by 1½ in. The skin over it was thoroughly adherent, but not ulcerated. On section the tumour was entirely composed of a congested, firm yellowish white tissue, dotted here and there with minute opaque yellow calcareous particles; the peripheral parts of the mass were broken up into nodules surrounded by hyperæmic fibrous tissue. At the inferior extremity of the tumour, and partly covered by it, there was a mass (2 in. by 1 in.) of caseous, slightly gritty tubercles, surrounded by firm tissue. A thickened tortuous caseous lymphatic vessel could be traced from the tumour to the left prescapular gland.

The tissues around the local tumour and the neighbouring glands were œdematous and congested.

Left Prescapular Gland.—Weighed 5 ozs., and measured 4 in. by 2 in. by 1⅛ in. On section it was caseous throughout and slightly gritty.

Prepectoral Glands.—A left prepectoral gland, the size of a small walnut, was deeply congested, and showed in the cortex a caseous mass affecting about one-third of the gland substance; in the less affected portion minute grey foci were seen. A second and deeper prepectoral gland was a little enlarged and showed one greyish focus. A third was not obviously affected.

Cervical Glands.—Two glands on the left side of the trachea, about midway, the size of broad beans, were caseous throughout. A corresponding gland on the opposite side was normal.

A gland a little anterior to the former, and slightly smaller, was slightly affected.

A gland about the level of the larynx, twice the size of a broad bean, was also tuberculous throughout.

Two small glands on the same side of the trachea (left), near the anterior opening of the thorax, were tuberculous; one showed a mass which occupied more than half the cortex, the other minute greyish-yellow foci.

Abdomen.

Omentum.—Free from fat. Showed on the inferior surface about a dozen tubercles, 1 to 2 mm. in diameter, a few of which were deeply congested; the surface in contact with the stomach and intestines was normal.

The peritoneal cavity contained about 6 ozs. of blood-stained fluid.

Spleen.—Weighed 4½ ozs. On the convex surface there was a firm flattened nodulated growth 7 mm. in diameter. On section the pulp was filled with nodules from 1 to 7 or 8 mm. in diameter. All the nodules were caseous in the centre, greyish around the margins: several contained calcareous grains.

Liver.—Weighed 4½ lbs. On both surfaces there was a considerable number of pearly nodules, ranging in size from 1 cm. downwards. On the convex surface the larger projecting nodules were flattened and had slightly overhanging margins; on the concave surface they were rounded. On section through the liver tubercles were moderately numerous in every part; these rarely exceeded 5 mm. in diameter. All the growths exhibited central caseation; a few only contained calcareous grains.

Hepatic Lymphatic Glands (four in number).—Two large, with nodular surfaces, and two small, weighed together 2½ ozs., and consisted throughout of masses and nodules of caseous, slightly gritty tuberculous tissue.

Kidneys.—The left weighed 9 ozs. and showed in the cortex near the surface twelve to fifteen slightly caseous tubercles ranging in size from a mustard seed to a good-sized shot. The tubercles appeared on the surface, but did not project. In the deeper parts of the cortex similar tubercles were seen, but these were few in number. In addition to the above there were three wedge-shaped areas occupying the whole thickness of the cortex, and projecting slightly above the surface, the largest being 1 cm. long by 0·5 cm. broad; these appeared to be formed of a number of small tubercles which had coalesced.

The right kidney (10 ozs.) showed similar, but less numerous tubercles, and two small wedge-shaped areas; the contiguous halves of two lobules in this kidney were grey in colour and anæmic.

Suprarenal Bodies.—The right contained in the medulla a grey tubercle the size of a pin's head; tubercle bacilli were found in a smear prepared from this. The left was normal.

Small Intestine.—Congested in parts (? hypostatic); no tubercles found.

Large Intestine.—No tubercles found.

Gastric Glands.—One gastric gland contained a caseating nodule 5 mm. in diameter. Two others showed minute foci.

Mesenteric Glands.—All the mesenteric glands, with about four exceptions, these being chiefly towards the posterior end, contained caseating nodules ranging in size from a small seed to a small pea.

Thorax.

Lungs.—With the thoracic glands attached, weighed 9 lbs. 4 ozs. The anterior lobes and anterior portions of the posterior lobes were completely consolidated, deeply congested and oedematous. The portions of the posterior lobes not entirely solid showed patches of consolidation alternating with oedematous and congested crepitant lung tissue.

The tissue of the lung was packed with tubercles and nodules ranging in size from 1 mm. or less up to 1 cm. in diameter. Those on the surface of the anterior lobes and the ventral surfaces of the posterior lobes formed greyish white projections, the majority rounded, but a few flattened and mushroomed. On section the nodules were grey and translucent, and contained centrally a caseous focus, in many cases gritty; the nodules had an irregular outline, and in several places had become confluent.

The fringes around the margins of the lungs were not hypertrophied, but showed at wide intervals a few small hard tubercles.

On the costal pleura, along the margins of the ribs, there were on each side from fifteen to twenty caseous tubercles 1 to 3 mm. in diameter; they occurred singly and in clusters of three and four.

Mediastinal Glands.—In the dorsal mediastinum there was a chain of large glands, seven in number, weighing together 9 ozs., the long posterior gland alone weighed 6 ozs. and measured $5\frac{1}{2}$ in. by $1\frac{3}{4}$ in. by $1\frac{1}{3}$ in. On section the glands were tuberculous throughout and slightly gritty; the two glands in the anterior part of the mediastinum showed more advanced caseation than those in the posterior.

Bronchial Glands.—The left bronchial gland weighed $1\frac{1}{2}$ ozs., the right about 1 oz.; both were caseous throughout.

A small gland in front of the right bronchial, the size of a French bean, was congested and showed a few grey foci.

Two peribronchial glands on the right side, the size of walnuts, were entirely caseous.

A small gland on the oesophagus contained a pea-sized caseous nodule; a second one showed minute grey foci.

The ventral mediastinal glands were slightly enlarged but not apparently tuberculous.

A gland in the caval fold of the pleura contained a caseous nodule the size of a large pea; a second gland in the same fold, but nearer the centre of the tendon, contained a nodule half the above size.

Diaphragm.—On the pleural surface of the diaphragm, on the left side, there was a group of four small caseous tubercles. Around the margins of the tendon there were a few delicate, very vascular outgrowths.

On the abdominal surface, dorsally on the right side, there were from fifteen to twenty flattened subperitoneal tubercles, about the size of wheat grains.

Pericardium.—The external surface presented some intensely red velvety granulations, and a number of small greyish-yellow tubercles.

The internal surface was normal.

The pericardial sac contained a small amount of yellow fluid.

Heart Muscle and Valves.—Normal.

Pancreas, Thymus, Thyroid, Tongue, Pharynx, Larynx, Tonsils.—Normal.

Various Lymphatic Glands.—Along the whole length of the abdominal aorta there was a chain of glands, eight to ten in number, ranging in size from a broad bean up to 2 in. in length. On section they all contained confluent caseous masses; in the case of the smaller glands replacing the whole of the gland tissue.

Iliac Glands.—A left iliac gland showed in the cortex two caseous nodules, 4 mm. in diameter; two small glands near this were not obviously affected.

A right iliac gland contained a caseous nodule 5 mm. in diameter.

A gland at the bifurcation of the aorta contained a similar nodule.

Right Submaxillary.—Contained a caseous tubercle the size of a No. 4 shot.

Right and Left Axillary, Right Prescapular, Right and Left Prepectoral, Right and Left Precrural, Right and Left Popliteal, Right and Left Gluteal, Right and Left Ischiatic, Right and Left Parotid, Right and Left Posterior Pharyngeal, Left Submaxillary, Vertebral.—Normal in size; no tubercle.

Microscopical Examination (Smear Preparations).

Prescapular Gland (Emulsion) *Right Suprarenal Body*	Tubercle bacilli.

Animals Inoculated.

With Emulsion of Prescapular Gland:—

G.P. 749. (S.) G.P. 750. (S.)	died in 37 days of general tuberculosis	
G.P. 751. (I.)	died in 21 days	of general tuberculosis.
G.P. 752. (I.)	died in 20 days	

CALF 130.

Subcutaneous.— Inoculation of an emulsion made from the inguinal and hepatic lymphatic glands and the spleen of Pig 12.

Dose.—10 cubic centimetres, containing approximately 9,000 tubercle bacilli.

Date.—October 2, 1903.

Weight.—October 6, 1903. 3 qrs. 8 lbs.

Killed.—January 15, 1904. 105 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on October 2nd, 1902.

A fortnight later there was a small patch of thickening at the seat of inoculation and the left prescapular gland was about twice the normal in size.

Three weeks after inoculation there was a flat swelling $3\frac{1}{2}$ in. by 2 in., adherent to the skin ; the prescapular gland measured $2\frac{1}{2}$ in. in length.

Seven weeks after inoculation the tumour measured 4 in. by $2\frac{1}{2}$ in. and the prescapular gland about 3 in. in length. Subsequently there was little change in the local conditions. The calf remained in good health during the experiment and gained in weight.

Tuberculin Tests.—January 6th, 1904. 96 days after inoculation. Positive. Rise of temperature 1·1° C.

January 13th, 1904, 103 days after inoculation. Positive. Rise of temperature 1·0° C.

Temperature.

Twelve days after inoculation the temperature rose to 39 8° C., the highest point reached during the experiment. Subsequently it remained very constant, varying only between 39·1 and 39·5° C.

Weights.

The calf was weighed every week and showed an almost steady uniform increase in weight.

October 6th, 1903 - -	0 cwt. 3 qrs. 8 lbs.
January 12th, 1904 - -	1 cwt. 1 qr. 26 lbs.
Gain in weight - - -	2 qrs. 18 lbs.
Rate of gain per week -	5·25 lbs.

Post-Mortem Examination.

Carcass.—Condition fair.

At the Seat of Inoculation.—On the left side of the neck there was a firm nodular thickening, 3 in. by $2\frac{1}{2}$ in. ; when removed and cut into the tumour was seen to be from $\frac{1}{2}$ to $\frac{3}{4}$ inch thick, and to consist of closely packed nodules 2 to 4 mm. in diameter, surrounded by fibroid tissue. The nodules were yellow in colour, and appreciably calcified ; they were for the most part separate, and some had coalesced to form large masses. The skin was adherent, but not ulcerated or infiltrated.

The growth was fixed to the aponeurosis of the superficial muscles. The muscles showed three small groups of nodules continuous with the main mass ; one proceeded from the anterior extremity, one from the posterior, and one from the under surface ; with the exception of these the tumour was wholly subcutaneous.

In the subcutaneous tissues over the inferior extremity of the prescapular gland there were two or three isolated calcareous tubercles ; similar tubercles occurred at the extremity of the gland, embedded in the muscle and fat ; calcareous tubercles, some subcutaneous, others intramuscular, were seen between the tumour and the mid line of the neck.

Left Prescapular Gland.—Weighed about an ounce, and measured $2\frac{1}{2}$ in. by $1\frac{1}{2}$ in. by $\frac{3}{4}$ in. On section it showed in the midst of the gland substance an oval mass (1 in. by $\frac{3}{4}$ in) which consisted of firm caseous tissue, slightly gritty in the central part, highly calcareous around the periphery ; in the cortex there were scattered calcareous nodules showing little caseous tissue. The lower part of the gland showed two similar nodules (the size of a pea) and minute scattered calcareous grains ; with these exceptions, this part of the gland had a normal appearance.

Prepectoral Glands (on left side).—The most superficial gland, the size of a walnut, showed beneath the capsule numerous elevated yellow nodules. On section it consisted throughout of firm caseating tissue, perceptibly gritty. A second gland, more deeply situated, and about half the size, contained a few yellow gritty foci—otherwise normal.

Cervical Glands.—A lower cervical gland on the left side, an inch in front of the manubrium sterni, showed caseous foci. A middle cervical gland on the same side showed similar foci.

Upper Cervical Glands.—Normal.

Right Middle and Lower Cervical Glands.—Normal.

Right and Left Axillary Glands.—Normal.

Right Prescapular Gland was enlarged, measuring 3 in. by $1\frac{1}{4}$ in. by $\frac{1}{2}$ in. ; on section soft and œdematous, with a patch of congestion (tuberculin test 41 hours previously) ; The subcutaneous tissues and muscles at seat of inoculation of tuberculin were slightly œdematous.

Right Prepectoral Glands.—Normal.

Abdomen.

Omentum.—Moderately fatty ; free from tubercles.

Peritoneum.—Everywhere normal.

Spleen.—Weighed 6 ozs. Normal on the surface ; contained in the pulp about a dozen yellow tubercles from 2 to 3 mm. in diameter ; the larger nodules only showed caseous and gritty foci centrally.

Liver.—Weighed 3 lbs. 6 ozs. On the anterior surface, beneath the capsule, but embedded in the parenchyma, there were two yellow nodules, one 2 mm., the other 4 mm. in diameter. The former was not caseous, the latter projected slightly, had a flattened surface, and was caseous in the centre.

On section the liver appeared normal throughout.

Hepatic Lymphatic Glands (three in number).—Two normal ; one contained a few calcareous grains.

Gall Bladder.—Normal.

Kidneys.—Each weighed 6 ozs. Both normal.

Suprarenal Bodies.—Normal.

Pancreas.—Normal.

Two glands between the stomach and the left pillar of the diaphragm were normal.

Gastric Glands, Mesenteric, Colic.—Normal.

Small Intestine, Large Intestine.—Normal throughout.

Tongue, Pharynx, Tonsils, Œsophagus, Posterior Pharyngeal Glands, Submaxillary, Parotid, Mastoid Glands.—On both sides normal.

Thorax.

Pericardium.—Contained a small amount of clear fluid.

Heart Muscle and Valves.—Normal.

Lungs.—Right cephalic lobe, normal.

Left cephalic lobe showed on the superior surface two soft vascular fibrous tufts, and in the substance beneath the pleura one grey translucent tubercle 2 mm. in diameter, and two or three minute grey points.

Left caudal lobe carried on the mediastinal surface a mushroomed nodule 4 mm. in diameter ; on this surface there were seen beneath the pleura two minute tubercles similar to those in the cephalic lobe.

On the superior surface of the right caudal lobe there was a small mushroomed growth; at the posterior margin of the lobe there was a firm nodule, 1 cm. in diameter, which occupied part of a lobule, and showed on section yellow foci; loosely attached to the free edge of this lobule there was a pinkish, fleshy fringe. Beneath the pleura in other parts of the lobe there were three caseous and gritty tubercles, 1 to 3 mm. in diameter; on section a similar tubercle was found in the substance.

The lung tissue, apart from the above-mentioned bodies, had a perfectly normal appearance.

Dorsal Mediastinal Glands.—The long caudal gland, not obviously enlarged, showed in the cortex here and there groups of yellow gritty foci. The two glands anterior to this showed scattered calcareous grains.

Left Bronchial Gland.—Not enlarged; contained a few groups of yellow gritty foci.

Right Bronchial Glands (two in number) were similarly affected.

Peribronchial Glands (on each side) also showed calcareous foci.

Ventral Mediastinal Glands.—Normal.

The glands just within the anterior opening of the thorax were normal.

Parietal pleura and parietal surface of diaphragm normal.

Larynx, Trachea, Mammary Gland, Supramammary Lymphatic Glands, Iliac Glands, Sacro-Iliac Glands, Lumbar Glands, Precrural Glands, Popliteal Glands, Gluteal Glands, Ischiatic Glands, Ovaries, Uterus, Fallopian Tubes, Thymus, Thyroid.—Normal.

Microscopical Examination.

Smears from:—

Prescapular Gland.—Tubercle bacilli.

Liver.—A few tubercle bacilli.

Spleen.—No tubercle bacilli.

Spleen.—No tubercle bacilli.

Animals Inoculated:—

An emulsion was prepared from the prescapular gland, and equal quantities inoculated into Guinea pigs 796 and 797 subcutaneously and 798 and 799 intraperitoneally; all died tuberculous in periods varying from 22 to 44 days.

PIG 98.

Subcutaneous.—Inoculation of culture from Pig 16.

Dose.—1·0 milligramme.

Date.—January 10, 1905.

Weight.—January 17, 1905. 1 qr. 16 lbs.

Died.—March 29, 1905, 78 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously in the wall of the abdomen on January 10, 1905.

Three weeks after inoculation there was a flat tumour 1 in. in greatest diameter; the right inguinal glands were moderately enlarged. Subsequently the local conditions showed very little alteration.

The animal was first noticed to be ill on March 1, 50 days after inoculation; the back was arched, the skin tight and wrinkled and the appetite poor. These signs continued and the animal got gradually worse; towards the end the respirations were visibly increased.

The temperature rose on the 9th day to 39·5° C.; with this exception the temperature was practically normal throughout.

Weights.

During the first three weeks there was a loss in weight of 2 lbs. then the weight steadily increased.

January 17, 1905 - - -	1 qr. 16 lbs.
February 7, 1905 - - -	1 qr. 14 lbs.
February 21, 1905 - - -	1 qr. 20 lbs.
March 7, 1905 - - -	1 qr. 24 lbs.
March 14, 1905 - - -	2 qrs. 0 lb.
March 28, 1905 - - -	2 qrs. 7 lbs.

Gain in weight, 19 lbs.

Post-Mortem Examination.

Carcass in very poor condition.

The Local Lesion was situated in the muscles on the right side of the abdomen a little anterior to the umbilicus; it was small and flat, measuring 1 in. by ¾ in. by ¼ in. and consisted of tough, waxy white caseous tissue interspersed with fine opaque white streaks of beginning calcification; the skin was slightly adherent at one point and was not infiltrated.

Superficial Inguinal Glands.—On the right side the one nearest the tumour was slightly enlarged and showed irregular caseous patches replacing about half the substance; other glands in this group appeared normal.

The glands on the left side were congested but otherwise normal.

Right Precrural Gland.—Normal.

Left Precrural contained a yellow caseous tubercle.

Iliac and Lumbar Glands were slightly congested; they showed no naked-eye evidence of tuberculosis.

Abdomen.

Omentum and Peritoneum.—Normal.

The Spleen was normal in size; it contained in the pulp from twenty to twenty-four greyish white nodules. ranging in size from a pin's head to a pea; the larger nodules had opaque yellow centres.

The Liver, slightly adherent to diaphragm, showed in the substance sparsely scattered grey tubercles the largest the size of a millet seed; the latter were slightly opaque in the centre.

Kidneys and Suprarenal Bodies.—Normal.

Thorax.

Heart.—Normal.

Lungs.—The lungs were deeply congested; the greater part of the middle lobes, portions of the cephalic lobes, and several lobules along the sternal borders of the caudal lobe were dark red, firm, and quite airless; they showed a few small tubercles and on close inspection grey spots were seen. In the crepitant portions of the lung there were moderately numerous yellow tubercles, the size of pins' heads; in the thin margin of the left caudal lobe there were several nodules, the largest the size of a pea, yellow and gritty in the centre. On the surface of the caudal lobes there were several small projecting nodules and numerous long strands of connective tissue, some of which grew from the surface of the nodules; the fringes around the margins of the caudal lobes were hypertrophied, congested, and contained, chiefly on the left side, firm caseating nodules, the largest the size of a pea.

The Bronchial and Mediastinal Glands were deeply congested, but were not obviously tuberculous.

Pleura.—In several places along the margins of the ribs the lymphatic fringes were slightly hypertrophied and contained little grey granules; here and there a slightly larger nodule was seen similar to those on the diaphragm.

Diaphragm.—The pleural surface showed numerous hard grey tubercles, the majority the size of millet seeds; these were situated at the points of attachment of congested connective tissue processes; the fringes around the margins of the tendon were considerably hypertrophied, and showed here and there caseous nodules, the largest the size of a split pea; some of these nodules were hæmorrhagic and were attached to the free extremity of a connective tissue strand.

On the Peritoneal Surface of the diaphragm there were numerous hard grey nodules, the largest the size of a wheat grain.

Tongue, Tonsils, and Pharynx.—Normal.

Submaxillary, Pharyngeal, and Cervical Glands.—Normal.

Prescapular, Prepectoral, and Popliteal Glands.—Normal.

Large and Small Intestines.—Normal.

Mesenteric Glands.—A gland in the anterior part of the mesentery contained a cheesy, gritty nodule, the size of a pea; in the terminal mesenteric gland there was a similar but much smaller nodule.

Microscopical Examination.

Left Precrural Gland.—Three tubercle bacilli seen.

Mesenteric Gland.—No tubercle bacilli.

Bronchial Gland.—No tubercle bacilli.

PIG 96.

Subcutaneous Inoculation of culture from Pig 16.

Dose.—0·1 milligramme.

Date.—January 10, 1905.

Weight.—January 17, 1905. 1 qr. 23 lbs.

Killed.—September 7, 1905. 240 days after inoculation.

Clinical History.

The pig was inoculated subcutaneously in the wall of the abdomen on January 10, 1905.

Three weeks after inoculation a small nodule was felt under the skin of the abdomen and the superficial inguinal glands were slightly enlarged. The tumour and glands increased a little in size and then diminished.

The animal was in fair condition when killed, and, beyond the fact that it was somewhat stunted in its growth, had not shown any signs of illness during the experiment.

The temperature was slightly raised on the 23rd and 24th days (maximum 39·6° C.), and again on the 67th day to 39·2°C.; otherwise the temperature was normal throughout.

Weights.

In the first ten weeks there was a gain in weight of 26 lbs.; in the 12th week there was a loss of 10 lbs., and for three weeks subsequently the weight showed slight fluctuations; during the rest of the experiment there was a slight, almost steady increase in weight.

January 17th, 1905 - -	1 qr. 23 lbs.
September 7th, 1905 - -	3 qrs. 19 lbs.
Gain in weight - - -	1 qr. 24 lbs.
Rate of gain per week - -	1·56 lbs.

Post-mortem Examination.

Local Lesion.—In the subcutaneous tissues to the right of the umbilicus, there was a softened, caseous, slightly gritty nodule, the size of a kidney bean; the skin was not adherent.

Superficial Inguinal Glands.—On the right side there were two moderately enlarged glands, which were caseous and softened practically throughout; the capsules of the glands were thickened; gritty particles could be detected in the caseo-pus when rubbed between the finger and thumb; in this group were also a pea-sized caseous gland, and two normal glands. On the left side one gland contained a nodule about 1 cm. in diameter with fibrous wall and caseo-purulent contents; a second gland showed on section a number of discrete yellow caseous nodules (largest size of wheat grain) embedded in fibroid glandular tissue; other glands on this side were normal.

Precrural Glands.—Normal.

Iliac Glands.—Normal.

Abdomen.

Omentum and Peritoneum.—Normal.

The Spleen contained three millet seed sized tubercles, with soft yellow centres, in one case gritty, and translucent fibrous margins.

Liver.—Embedded in the substance of the liver just under the capsule a moderate number of nodules was seen (about four dozen counted on the anterior surface, and three dozen on the posterior); the nodules ranged in size from about 1·5 up to 7 and 8 mm. in diameter; all, with the exception of one or two of the smallest, which were grey and translucent throughout, had fibrous capsules and soft viscid caseous contents; in the latter fine gritty particles could be detected; on section similar nodules were seen in the depth of the liver.

Portal Glands.—In the small omentum there was a group of four enlarged glands, the capsules of which were adherent; on section the gland tissue was completely replaced by soft, cheesy, slightly gritty, caseous material; the capsules of the glands were much thickened.

Cœliac Glands were moderately enlarged and contained discrete yellow caseous nodules the size of wheat grains; two others contained similar nodules, but these in one or two places had run together to form soft caseous collections.

Lumbar Glands.—A gland on the left side of a lumbar vertebra showed about half its substance caseous and softened.

Kidneys and Suprarenal Bodies.—Normal.

Thorax.

Heart and Pleura.—Normal.

The Lungs contained sparsely scattered hard tubercles, the largest the size of a wheat grain; the tubercles were translucent and fibrous, and most had opaque yellow centres, which in several were quite calcareous; the tubercles were most numerous in the right cephalic and middle lobes, and many of these were translucent throughout; the lungs were crepitant and there were no areas of consolidation; in the fringes around the margins of the right caudal lobe were several tubercles.

The Bronchial Glands on each side were moderately enlarged; on section their capsules were thickened and their substance almost completely replaced by soft, cheesy, slightly gritty, caseous material.

The Dorsal Mediastinal Glands were normal.

Ventral Mediastinal Glands.—One, the size of a Barcelona nut, was caseous throughout; another similar in size showed discrete yellow caseous nodules, each about 2 mm. in diameter; the gland tissue between the nodules showed an increase in interstitial connective tissue.

A small gland just within the entrance to the thorax contained two yellow millet seed sized tubercles.

Diaphragm.—On the left side of the diaphragm projecting under the peritoneum were three or four hard translucent nodules, the largest the size of a small kidney bean; the latter had a soft, yellow centre.

On the Serous Surface of the stomach near the œsophagus there were about half-a dozen nodules, the largest the size of a wheat grain; one had a soft caseous centre the others were translucent throughout.

A gland near the angle of the jaw on the left side contained a pea-sized firm caseous nodule.

The right Pharyngeal Gland contained a small collection of viscid caseo-pus.

Other Glands in the Neck were normal.

Intestines.—Normal.

Mesenteric Glands.—Two glands in the anterior part of the mesentery contained each a pea-sized softened caseous nodule; a gland towards the root of the mesentery showed two pinhead-sized caseous foci.

Colic Glands.—Normal.

Prescapular and Prepectoral Glands.—Normal.

Testicles.—Normal.

Microscopical Examination (Smear Preparations).

Portal Gland (Emulsion for G.P.).—No tubercle bacilli.

Bronchial Gland (Emulsion for G.P.).—No tubercle bacilli.

Animals Inoculated,
With Tissue Emulsions.

Guinea-pig 1679, inoculated intraperitoneally with an emulsion of a portal gland, died of general tuberculosis in 38 days.

Guinea-pig 1680, inoculated intraperitoneally with an emulsion from a bronchial gland, died of general tuberculosis in 33 days.

With Culture.
Rabbits.

Number of Rabbit.	Initial Weight in grammes.	Final Weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
558	1,620	1,090	1·0 mg.	Subcutaneous	Died, 81 days	General tuberculosis.
559	1,510	1,140	0·1 mg.	Subcutaneous	Died, 137 days	General tuberculosis.
560	1,310	1,000	0·01 mg.	Subcutaneous	Died, 106 days	General tuberculosis.
561	1,220	1,100	0·001 mg.	Subcutaneous partly intra-peritoneal.	Died, 50 days	General tuberculosis.

CALF 230.

Subcutaneous Inoculation of culture from Pig 16.

Dose.—50 milligrammes.

Date.—January 10, 1905.

Weight.—January 17, 1905. 3 qrs. 3 lbs.

Died.—February 13, 1905. 34 days after inoculation.

Clinical History.

The calf was inoculated subcutaneously on the left side of the neck on January 10, 1905.

Two days later a small swelling was felt; this rapidly increased in size, and on January 30, 20 days after inoculation, attained its maximum, measuring 6 in. in length, 3 in. in breadth, and about ¾ in. in greatest thickness; on this date the left prescapular gland measured 3¼ in. in long diameter and a prepectoral gland was the size of a walnut. The skin over the upper part of the tumour then broke down, discharging serous fluid.

On February 6 the respirations were noticed to be increased and the animal looked ill and thinner.

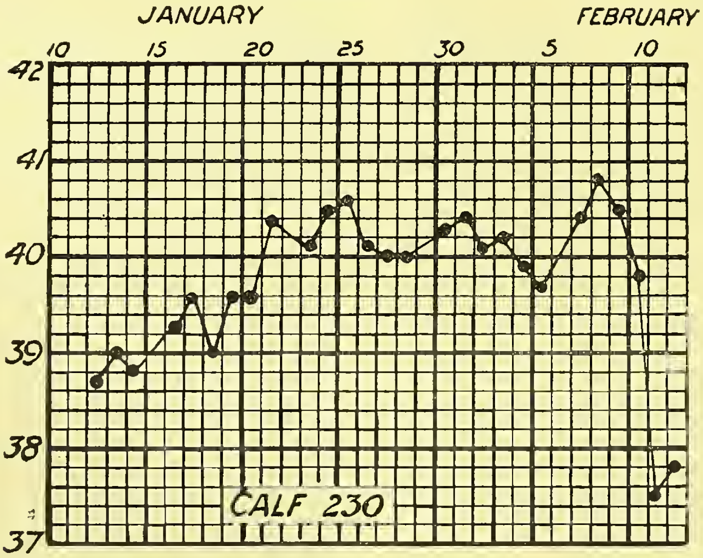
The symptoms rapidly increased in severity; the respirations became quicker, and finally laboured, and the animal visibly lost flesh. Death took place on February 13, 1905, 34 days after inoculation.

Weights.

There was a steady loss in weight.

January 17, 1905 - - -	3 qrs. 3 lbs.
February 7, 1905 - - -	3 qrs. 0 lb.
February 13, 1905 - - -	2 qrs. 18 lbs.
Loss in Weight - -	13 lbs.
Rate of Loss per Week -	3·36 lbs.

Temperature



Post-Mortem Examination.

Carcass.—Emaciated.

Seat of Inoculation.—At the seat of inoculation, on the left side of the neck, there was a flat elongated tumour 6 in. by 2¾ in. by 1 in. On section it consisted of dense yellow caseo-necrotic tissue, adherent to the skin, and slightly infiltrating the muscles; in the upper part of the tumour there was an empty cavity 2 in. by 1½ in., whose walls were formed by breaking down caseated tissue; the skin over the cavity was infiltrated up to the surface, and in the central part ulcerated and necrosed.

The tissues around the tumour were slightly œdematous.

Left Prescapular Gland.—Weighed about 2 ozs., and measured 3 in. by 1½ in. by 1 in. On section it was composed throughout of dense pinkish yellow caseated tissue.

Left Prepectoral Glands.—The rounded prepectoral gland was three quarters of an inch in diameter, and on section resembled the prescapular. The kidney-shaped prepectoral and a small gland near it were œdematous, but were otherwise unaltered to the naked eye.

Cervical Glands.—A mid cervical gland on the left side showed the cortex partly caseous. All other cervical glands contained discrete caseous tubercles.

Abdomen.

Omentum.—Was quite devoid of fat. On the under surface there were a few pinhead-sized tubercles.

Spleen.—8 ozs. Normal on the surface; substance packed with firm yellow tubercles, ranging in size from 1·5 millimetres to about 3 millimetres in diameter.

Liver.—Weighed 3 lbs., and was not apparently enlarged. The substance was evenly peppered with grey tubercles, ranging in size from a mere point to that of a pin's head; the larger tubercles were slightly opaque in the centre.

Gall Bladder.—There were two or three yellow tubercles under the mucous membrane.

Portal Glands.—Were moderately enlarged. They showed in their cortices firm grey tissue, closely studded with irregular caseous foci.

Pancreas.—In the substance there was a minute yellow point.

Kidneys.—Right kidney showed in the cortex moderately numerous opaque grey miliary tubercles, some of which on section were seen to be slightly elongated in a radial direction. Left kidney similarly affected. No tubercles were seen in the medullary zones.

Suprarenal Bodies.—In the cortex of each suprarenal there was a greyish white focus.

Coeliac Glands.—Were enlarged, and resembled on section the portal.

Thorax.

Heart.—Muscle pale and flabby; cavities filled with ante-mortem blood clot; on the endocardium of the right ventricle there was a mustard seed-sized pearly tubercle.

Parietal Pleura and Diaphragm.—Normal.

Lungs.—Were moderately closely beset with opaque miliary tubercles; the right, middle and caudal lobes were solid, dark red in colour, and sank in water (animal found dead on right side); the right cephalic and the left lobes showed patches of consolidation, mainly small, which surrounded small groups of tubercles.

Mediastinal Glands.—The dorsal mediastinal glands showed a moderate degree of enlargement. On section they showed in the cortex firm grey tissue in a state of early caseation (they resembled the portal glands); the medullæ were congested.

Bronchial Glands, Peribronchial Glands.—Were enlarged, and on section resembled the mediastinal.

The gland below the trachea was enlarged, congested and œdematous; no foci of caseation seen.

The ventral mediastinal glands each showed one or two caseous tubercles.

Alimentary Tract.

Tonsils.—Contained a few caseous tubercles. On the superior surface of the soft palate there were two or three small ulcerated caseous nodules.

Small Intestine.—Under the mucous membrane of the small intestine there were sparsely scattered caseous tubercles; these were most numerous in the ileum.

Large Intestine.—Normal.

Gastric Glands.—Were enlarged, and composed practically throughout of grey translucent tissue, studded with minute opaque foci.

Mesenteric Glands.—The glands in the anterior three-fourths of the mesentery showed here and there opaque miliary tubercles; those in the posterior quarter were more severely affected.

Various Lymphatic Glands.

Colic Glands.—Several contained miliary tubercles.

Submaxillary Glands, Pharyngeal Glands were closely beset with caseous tubercles.

Gluteal Glands, Popliteal Glands, Pudic Glands, Precrural Glands, Iliac Glands, Sacro-Iliac Glands, Right Prescapular Gland, Right Axillary Gland.—Contained a moderate number of caseous tubercles.

Right Prepectoral Glands.—One right prepectoral gland showed a caseous focus.

Lumbar Glands.—Showed numerous caseous foci.

Ischiatic Glands.—Appeared normal.

Testicles, Eyes.—Normal.

Microscopical Examination.

Tonsils.—Tubercle bacilli moderately numerous.

$\left.\begin{array}{l}\textit{Soft Palate}\\ \textit{Pancreas}\\ \textit{Gall Bladder}\end{array}\right\}$ Tubercle bacilli numerous.

Heart.—One tubercle bacillus.

$\left.\begin{array}{l}\textit{Right Suprarenal Body}\\ \textit{Left Suprarenal Body}\end{array}\right\}$ A few tubercle bacilli in each.

Tables showing the Virulence for

RABBITS

# of Cultures derived from the different Animals in the Report on Modification Experiments.

Strains of Virus B. I.

RABBITS INOCULATED WITH CULTURE DERIVED FROM MONKEY 76.

The culture used was the 5th generation, 20 days old, and had been in cultivation 91 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
189	3,560	1,920	0·1 mg.	Intravenous	24 days (died)	Lungs packed with grey miliary tubercles with yellow centres; numerous tubercles in the kidneys and numerous minute grey foci in the liver and spleen; latter organ much enlarged; all the lymphatic glands contained caseous foci.
192	2,930	1,870	1·0 mg.	Intravenous	16 days (died)	General tuberculosis as in Rabbit 189, but tubercles more minute.
190	3,050	1,970	1·0 mg.	Intraperitoneal	23 days (died)	Omentum retracted, slightly thickened and in a state of early caseation. Parietal and visceral peritoneum uniformly and closely covered with small opaque tubercles. Liver and spleen closely beset with minute tubercles; kidneys showed fairly numerous tubercles; lungs packed with miliary caseating tubercles; all the lymphatic glands were tuberculous, the sternal glands being caseous throughout
191	2 830	1,600	1·0 mg.	Intraperitoneal	32 days (died)	General tuberculosis as in Rabbit 190, but tubercles a little larger.
193	3,580	2,400	1·0 mg.	Subcutaneous	47 days (died)	Caseous tumour between scapulæ; skin over it ulcerated; neighbouring glands caseous; lungs crowded with caseating nodules; cephalic lobes solid; numerous tubercles in liver and kidneys; spleen greatly swollen, packed with caseous nodules, up to 5 mm. in diameter; most of the lymphatic glands were affected.
194	2,960	2,360	1·0 mg.	Subcutaneous	63 days (died)	General tuberculosis; the local lesion was situated between the scapulæ and was large, firm and caseous.

RABBITS INOCULATED WITH CULTURE DERIVED FROM MONKEY 80.

The subculture used was the 6th generation, 20 days old, and had been in cultivation 177 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
216	920		0·1 mg.	Intravenous	8 days (died)	Cause of death not ascertained.
217	1,370	970	0·1 mg.	Intraperitoneal	37 days (died)	General tuberculosis; lungs packed with confluent caseating nodules.
218	1,220	920	1·0 mg.	Intraperitoneal	47 days (died)	General tuberculosis; tubercles were fairly numerous on the mesocolon, but sparsely scattered; on the parietal peritoneum; there was a bean-sized caseous nodule in the abdominal wall (the inoculation was partly subcutaneous).
219	1,100	980	1·0 mg.	Subcutaneous	42 days (died)	Caseous abscess between the scapulæ; general tuberculosis, lungs severely affected.
220	1,000	700	1·0 mg.	Subcutaneous.	49 days (died)	Firm caseous tumour breaking down in the centre, on left scapula; neighbouring glands caseous; lungs enlarged and composed almost entirely of caseating nodules; numerous tubercles in spleen and kidneys; liver packed with firm grey nodules; there were a few minute tubercles in the omentum and on the mesentery.

RABBITS INOCULATED WITH CULTURE DERIVED FROM MONKEY 82.

The subculture used was the 5th generation, 20 days old, and had been in cultivation 169 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose	Method of Inoculation.	Duration of Life.	Result.
221	1,050	850	0·1 mg.	Intravenous	26 days (died)	Lungs packed with miliary tubercles; numerous tubercles in kidneys and numerous very minute grey foci in liver and spleen.
222	1,320	950	0·1 mg.	Intraperitoneal	46 days (died)	Omentum thickened and caseating; sparsely scattered caseous tubercles on parietal peritoneum; very numerous tubercles on mesentery and meso-colon; a few minute tubercles in liver and spleen and a few miliary caseating tubercles in each kidney; lungs almost completely consolidated, being filled with confluent caseating nodules.
223	1,150	1,170	1·0 mg.	Intraperitoneal	91 days (died)	Omentum thickened and composed of lobulated masses of firm caseating tissue, the margins of which were darkly pigmented. Yellow caseous tubercles with pigmented margins were sparsely scattered over the surface of the parietal and visceral peritoneum. The spleen and liver showed a few flattened tubercles on the surfaces: none were seen in the depth. The kidneys were closely beset with caseating nodules. The lung tissue was extensively replaced by irregular caseating masses, in some cases softened. Sternal glands caseous throughout; the bronchial showed caseous foci. The diaphragm was thickened and caseating and there were some flattened growths on the parietal pleura.
224	1,200	870	10·0 mg.	Intraperitoneal	21 days (died)	General tuberculosis; tubercles very numerous on the peritoneum and in all the organs.
225	1,250	1,910	1·0 mg.	Subcutaneous	114 days (died)	Caseous ulcer between the shoulders; neighbouring glands large and caseous; moderate number of tubercles in omentum; lungs severely affected, consisting almost throughout of caseating tuberculous masses; a few miliary tubercles in each kidney; no tubercles in liver or spleen. The abdominal and thoracic lymphatic glands appeared normal.

RABBITS INOCULATED WITH CULTURE DERIVED FROM BABOON 64.

The subculture used was the 4th generation, 22 days old and had been in cultivation 99 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
857	1,760	1,200	0·1 mg.	Intravenous	21 days (died)	Lungs closely beset with grey miliary tubercles caseous in the centre ; thoracic glands enlarged and oedematous, showed patches of caseations in the cortices ; spleen greatly enlarged and swollen, pulp speckled with grey foci ; liver closely beset with grey foci ; numerous small grey tubercles in each kidney ; most of the lymphatic glands contained caseous foci and there were minute grey points in the heart muscle.
858	1,630	1,070	0·1 mg.	Intraperitoneal	15 days (died)	Omentum thickened and caseating ; parietal and visceral peritoneum covered with minute grey tubercles ; lungs contained numerous grey tubercles with slightly opaque centres ; spleen slightly enlarged; liver contained psorosperm nodules and minute grey points ; a few minute tubercles just visible in the kidneys.
859	1,060	880	0·1 mg.	Intraperitoneal	16 days (died)	General tuberculosis resembling that in Rabbit 858 ; lungs were packed with grey tubercles, with opaque centres, which caused almost complete consolidation of all the lobes.
860	1,000	850	1·0 mg.	Subcutaneous	91 days) (killed, dying)	General tuberculosis.

RABBITS INOCULATED WITH CULTURE DERIVED FROM PIG 16.

The culture used was the 10th generation 20 days old, and had been in cultivation 359 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
159	1,700	1,200	1·0 mg.	Intravenous	21 days (died)	Lungs closely beset with miliary tubercles just beginning to caseate ; bronchial glands showed caseous nodules ; the liver and a greatly swollen spleen contained innumerable minute grey foci ; there were moderately numerous early tubercles in each kidney.
158	1,000	1,070	1·0 mg.	Intraperitoneal	62 days (died)	General tuberculosis ; there was a large caseous nodule in the muscle wall : inoculation probably partly intra muscular.

Rabbits Inoculated with Culture derived from Pig 96.

The culture used was the 4th generation, 22 days old, and had been in cultivation 88 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
558	1,620	1,090	1·0 mg.	Subcutaneous	81 days (died)	Caseous ulcer ; widespread generalised tuberculosis ; lungs filled with caseating nodules.
559	1,510	1,140	0·1 mg.	Subcutaneous	137 days (died)	Caseous ulcer ; inguinal and axillary glands enlarged and caseous ; lungs enlarged and extensively tuberculous ; kidneys contained numerous nodules up to 12 mm. ; spleen normal ; there were two doubtful tubercles in the liver : many lymphatic glands were tuberculous ; the left lobe of the thyroid was almost entirely caseous, and there was a caseous nodule in that of the right.
560	1,310	1,000	0·01 mg.	Subcutaneous	106 days (died)	Caseous ulcer ; general tuberculosis ; the lungs were crepitant and contained only a moderate number of discrete nodules ; there was one tubercle in the spleen and a doubtful tubercle in the liver.
561	1,220	1,100	0·001 mg.	Subcutaneous	50 days (died)	Widespread generalised tuberculosis ; the omentum was thickened and caseating, and there were scattered tubercles on the peritoneum ; the inoculation was therefore partly intraperitoneal.

Rabbits Inoculated with Culture derived from the Spleen of Baboon 66.

The culture used was the 4th generation, 20 days old ; the total age of the culture was 149 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
984	1,580	1,180	1·0 mg.	Intravenous	Died, 14 days	Miliary tuberculosis of lungs ; numerous minute tubercles in spleen (very large), liver and kidneys.
985	1,410	1,090	0·1 mg.	Intravenous	Died, 26 days	General tuberculosis ; tubercles very numerous in all the organs.
987	1,210	870	1·0 mg.	Intraperitoneal	Died, 12 days	General tuberculosis ; omentum thickened and caseating ; parietal and visceral peritoneum closely covered with minute tubercles ; numerous minute grey foci in liver and spleen ; a few in each kidney ; moderate number of minute tubercles in the lungs ; tuberculosis of abdominal and thoracic lymphatic glands.
986	1,030	—	0·1 mg.	Intraperitoneal	Died, 1 day	Severe coccidiosis of liver.

Strain of Virus B. II.

RABBITS INOCULATED WITH CULTURE DERIVED FROM GUINEA-PIG 1,735, THE LAST OF A SERIES OF 24 GUINEA-PIGS, THE FIRST OF WHICH WAS INOCULATED WITH THE ORIGINAL MATERIAL OF VIRUS B. II.

The Culture used was the 9th generation, 12 days old ; the total age of the Culture was 300 days.

Number of Rabbit	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
1,027	1,870	1420	1·0 mg.	Intravenous	Died, 15 days	General tuberculosis.
1,024	1,020	950	0·1 mg.	Intravenous	Died, 18 days	General tuberculosis.
1,025	1,020	920	0·1 mg.	Intravenous	Died, 18 days	General tuberculosis.
1,026	1,150	820	1·0 mg.	Intraperitoneal	Died, 12 days	General tuberculosis ; tubercles everywhere numerous and very minute.
1,028	960	970	0·1 mg.	Intraperitoneal	Died, 29 days	General tuberculosis: numerous tubercles on peritoneum and mesentery ; liver and spleen packed with grey foci ; caseous tubercles in many of the lymphatic glands.

Strains of Virus B IV.

RABBITS INOCULATED WITH CULTURE DERIVED FROM CHIMPANZEE 4.

The culture inoculated was the third generation, 19 days old ; the total age of the culture was 46 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
166	2,150	1,460	0·1 mg.	Intravenous	28 days (died)	General tuberculosis; lungs closely packed with miliary tubercles with caseous centres ; liver and spleen closely beset with minute tubercles; each kidney contained fairly numerous miliary tubercles ; many of the lymphatic glands showed minute caseous foci.
167	2,320	1,750	0·1 mg.	Intraperitoneal	39 days (died)	General tuberculosis; the peritoneum was covered with tubercles, and there were numerous tubercles in the lungs, spleen, and liver ; the kidneys contained only a few.
168	2,400	1,950	1·0 mg.	Intraperitoneal	61 days (died)	General tuberculosis ; there was a small caseous nodule in the subcutaneous tissues, and a larger one in the muscles of the abdominal wall.
169	2,660	1,890	1·0 mg.	Intraperitoneal	24 days (died)	General tuberculosis ; tubercles were very numerous on the peritoneum and in the lungs ; the spleen and liver were peppered with minute grey foci, and there were fairly numerous tubercles in each kidney: the sternal glands were caseous throughout ; the bronchial glands contained caseous tubercles.
170	2,250	1,550	1·0 mg.	Subcutaneous	74 days (died)	General tuberculosis ; lungs severely affected.
171	2,000	1,640	1·0 mg.	Subcutaneous	94 days (died)	Local caseous ulcer ; adjacent gland caseous ; lungs enlarged and composed practically throughout of caseating nodules ; there were very numerous nodules in the kidneys and none in the liver or spleen ; several lymphatic glands contained caseous tubercles.

Rabbits Inoculated with Culture derived from Chimpanzee .

The culture inoculated was the 3rd generation, 21 days old ; the total age of the culture was 49 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
240	1,360	1,040	0·1 mg.	Intravenous	18 days (died)	Lungs, œdematous and congested, packed with miliary tubercles ; spleen (enlarged) and liver speckled with minute grey foci, numerous miliary caseating tubercles in each kidney. There were a few tubercles on the endocardium of the right ventricle. The thoracic glands showed caseous areas in the cortices ; the iliac and lumber glands caseous foci ; numerous tubercle bacilli were found in the marrow.
242	840	720	0·1 mg.	Intraperitoneal	31 days (died)	General tuberculosis ; all the organs were affected, the lungs and kidneys severely.
239	1,620	1,140	1·0 mg.	Intraperitoneal	20 days (died)	General tuberculosis ; there were very numerous tubercles on the peritoneum (parietal and visceral) and in all the organs.
244	680	570	1·0 mg.	Intraperitoneal	19 days (died)	General tuberculosis ; similar to that in Rabbit 239.
241	1,260	1,070	1·0 mg.	Subcutaneous	77 days (died)	General tuberculosis ; the local lesion was a large baggy cyst, situated over the left scapula, containing blood-stained serous fluid and large caseo-necrotic masses ; the lungs were enlarged and extensively tuberculous.
243	730	910	1·0 mg.	Subcutaneous	62 days (died)	Local caseous ulcer ; inguinal and axillary glands much enlarged and extensively caseous ; tuberculosis of all the organs and most of the lymphatic glands ; the lungs were almost completely consolidated.

Rabbits Inoculated with Culture derived from Chimpanzee 8.

The culture inoculated was the 6th generation, 20 days old ; the total age of the culture was 84 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
320	1,210	1,000	0·1 mg.	Intravenous	16 days (died)	General tuberculosis.
321	1,190	1,100	1·0 mg.	Intraperitoneal	14 days (died)	General tuberculosis (uncomplicated) ; there were very numerous minute tubercles on the peritoneum and in all the organs. Tubercle bacilli were found in the bone marrow.
323	970	850	0·1 mg.	Intraperitoneal	5 days (died)	No sign of disease.
324	560	470	0·1 mg.	Subcutaneous	64 days (died)	Caseous ulcer ; adjacent glands caseous ; the lungs contained numerous caseating tubercles and irregular caseous patches ; there were sparsely scattered tubercles in the kidneys ; no tubercles were seen in the spleen or liver.
322	1,030	860	1·0 mg.	Subcutaneous	53 days (died)	General tuberculosis.
325	850	670	1·0 mg.	Subcutaneous	8 days (died)	Caseous patch in subcutaneous tissues of abdominal wall ; inguinal slightly enlarged and congested ; no sign of disease elsewhere. Microscopical. — Tubercle bacilli numerous in an inguinal gland ; none found in the spleen.

Rabbits Inoculated with Culture derived from Chimpanzee 10.

The culture inoculated was the 3rd generation, 21 days old ; the total age of the culture was 60 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
658	1,500	1,240	0·1 mg.	Intravenous	20 days (died)	Lungs packed with miliary tubercles ; liver and spleen peppered with minute grey points ; kidneys contained numerous submiliary tubercles ; there were numerous tubercles on the surface of the left ventricle and endocardium of the right.
660	1,780	1,370	1·0 mg.	Intraperitoneal	18 days (died)	General tuberculosis ; the tubercles were very numerous on the peritoneum and in all the organs.
662	1,520	1,290	0·1 mg.	Intraperitoneal	32 days (died)	General tuberculosis ; the lungs were closely beset with caseating miliary tubercles which caused almost complete consolidation of the anterior lobes ; the tubercles on the peritoneum and on the liver and spleen were not so numerous as in Rabbit 660.
664	1,570	1,240	0·01 mg.	Intraperitoneal	41 days (died)	General tuberculosis ; the lungs were extensively consolidated and composed practically throughout of greyish red translucent tissue containing irregular yellow caseous foci ; there were fairly numerous miliary tubercles in all the other organs and most of the lymphatic glands were tuberculous.
659	1,470	1,090	1·0 mg.	Subcutaneous	96 days (died)	Local ulcer ; tuberculosis of all the lymphatic glands and all the organs, except the spleen ; the lungs were severely affected and were almost quite solid. Each lobe of the thyroid contained an opaque tubercle.
661	1,430	1,430	0·1 mg.	Subcutaneous	85 days (died)	Large local ulcer ; nearest glands caseous ; lungs and kidneys closely beset with caseous nodules ; the liver appeared normal ; the spleen contained one tubercle ; most of the lymphatic glands were tuberculous ; the submaxilliary salivary gland contained a yellow tubercle and there was one tubercle in each lobe of the thyroid.
663	1,590	1,300	0·01 mg.	Subcutaneous	100 days (died)	General tuberculosis similar to that in Rabbit 659.

RABBITS INOCULATED WITH CULTURE DERIVED FROM CHIMPANZEE 16.

The culture inoculated was the 5th generation, 21 days old; the total age of the culture was 89 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
948	1,120	940	1·0 mg.	Intravenous	14 days (died)	General tuberculosis of the usual type for this mode of inoculation.
949	1,120	850	0·1 mg.	Intravenous	16 days (died)	
950	1,200		1·0 mg.	Intraperitoneal	9 days (died)	Slight tuberculosis of omentum and peritoneum and ventral mediastinal glands.
951	1,020	890	0·1 mg.	Intraperitoneal	42 days (died)	General tuberculosis; numerous tubercles in liver and spleen; the lungs were closely filled with grey tubercles, caseous in the centre; the cephalic lobes were quite airless.
954	1,570	1,220	·001 mg.	Intraperitoneal	54 days (killed)	Omentum thickened and considerably caseated; the spleen and liver contained moderately numerous submiliary tubercles; a few tubercles up to a hemp seed in each kidney; the lungs were large and filled with irregular translucent nodules with caseous foci.
955	1,200	820	·001 mg.	Intraperitoneal	45 days (died)	Omentum greatly thickened; scattered nodules up to a hemp seed on the visceral and parietal peritoneum; spleen showed moderately numerous yellow miliary tubercles, the liver a few; scattered grey tubercles with caseous centres in the kidneys; the lungs did not collapse and were fairly closely filled with irregular caseous areas. Mediastinal glands enlarged and caseous.
952	1,050	1,270	1·0 mg.	Subcutaneous	54 days (killed)	Softening caseous tumour the size of a pigeon's egg; three yellow miliary tubercles in the spleen; liver normal; kidneys showed nodules up to a small pea in size with little central caseation; the lungs were congested and contained numerous discrete tubercles with caseous centres; caseous foci in mediastinal, bronchial, and left iliac gland.
953	1,020	1,500	0·1 mg.	Subcutaneous	54 days (killed)	Shallow ulcer, 1¼in. in diameter, covered with a caseous scab; left axillary and inguinal glands partly caseous; spleen and kidneys each showed a single tubercle; liver normal; lungs are congested and show sparsely scattered translucent tubercles up to a millet seed.

RABBITS INOCULATED WITH CULTURE DERIVED FROM THE LUNG OF BABOON 62.

The culture used was the 5th generation, 20 days old ; the total age of the culture was 91 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Methods of Inoculation.	Duration of Life.	Post-mortem Result.
874	2,100	1,630	0·1 mg.	Intravenous	Died, 19 days	General tuberculosis of the usual type.
873	1,850	1,360	0·1 mg.	Intraperitoneal	Died, 10 days	Omentum thickened ; fairly numerous tubercles on peritoneum ; a few minute grey tubercles in spleen and the kidneys ; moderately numerous tubercles in liver and lungs ; ventral mediastinal glands partly caseous.
872	1,500	1,950	1·0 mg.	Subcutaneous	Killed, 108 days	Small clean ulcer in skin, soft caseous nodules in nearest glands ; spleen, liver and left kidney normal, right kidney showed two grey tubercles with caseous centres ; lungs crepitant but slightly congested, contained scattered superficial nodules up to 4 mm. in diameter, soft and caseous in the centre, also a nodule 1 in. by 1½ in. filled with caseo-pus ; other lymphatic glands normal.

RABBITS INOCULATED WITH CULTURE DERIVED FROM THE LUNG OF DOG 92.

The culture used was the 4th generation, 22 days old ; the total age of the culture was 113 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
1009	1,400	990	1·0 mg.	Intravenous	Died, 14 days	General tuberculosis of the usual acute type.
1010	1,200	750	0·1 mg.	Intravenous	Died, 21 days	Ditto.
1013	1,250	620	1·0 mg.	Intraperitoneal	Died, 20 days	General tuberculosis ; the lungs were crepitant and contained only a moderate number of small tubercles
1011	1,200	850	0·1 mg.	Intraperitoneal	Died, 26 days	General tuberculosis ; tubercles were numerous in all the organs.
1012	1,200	1,540	1·0 mg.	Subcutaneous	Killed, 40 days	Soft caseous subcutaneous tumour size of pigeon's egg ; curdy caseous nodules in nearest glands ; one tubercle in the spleen and a few in each kidney ; a few scattered tubercles in the lungs.

Rabbits Inoculated with Culture Derived from Dog 18.

The culture used for this series was the 9th generation, 20 days ; the total age of the culture was 249 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
410	1170	1730	0·1 mg.	Intravenous	97 days (killed)	The lungs were pinkish and collapsed normally; they showed evenly distributed throughout a moderate number of firm grey miliary tubercles with opaque centres. The left kidney showed in the depth of the cortex two grey miliary tubercles, slightly opaque in the centre. In the right kidney there was a similar tubercle and one of the calyces was filled with curdy caseo-pus. Other organs and all lymphatic glands were normal.
409	1530	1570	1·0 mg.	Intraperitoneal	97 days (killed)	The omentum contained scattered softened yellow nodules, up to 3 mm. in diameter and fairly numerous opaque pinhead sized tubercles; there were a few tubercles on the surface of the large intestine, liver and spleen, and a few groups of small tubercles on the mesentery between the vessels. The lungs contained a few grey miliary tubercles, slightly opaque in the centre, occurring chiefly in groups. The right kidney contained two or three minute grey tubercles in the cortex. The liver and spleen were normal on section. All lymphatic glands were normal.
411	1100	980	0·1 mg.	Intraperitoneal	68 days (died)	Omentum, not thickened, showed numerous spherical pearly white tubercles, smaller than pins' heads. There was a small number of similar tubercles on the mesentery, meso-colon and serous surfaces of the large intestine ; on the mesentery they formed clusters in the centres of the areas between the vessels. The lungs collapsed normally and contained a few grey tubercles, singly and in small groups, with very little central necrosis. Other organs and all the lymphatic glands were normal. The mucous membrane of the stomach was ulcerated.
408	1500	2170	1·0 mg.	Subcutaneous	97 days (killed)	In the subcutaneous tissues of the abdominal wall there was a thin-walled cyst, the size of a sparrow's egg, filled with thick creamy caseo-pus. Nearest glands normal. The right caudal lobe showed under the pleuræ a flattened congested nodule 5 mm. in diameter with a yellow caseous centre ; there was besides a pin-head sized grey tubercle opaque in the centre. There was no sign of tuberculosis elsewhere.
412	1020	1730	0·1 mg.	Subcutaneous	97 days (killed)	In the skin of the abdomen there was a small ulcer communicating with a small cavity with fibrous walls and caseo-purulent contents. In the right lobe of the lung there was a small irregular grey nodule containing minute caseous points. Other organs and all lymphatic glands were normal.
890	1520	1620	10·0 mg.	Intraperitoneal	83 days (killed)	Omentum contained translucent miliary tubercles with calcareous centres : a few similar tubercles on peritoneum : spleen and liver normal : three caseous miliary tubercles in the kidneys ; the lungs, pink and crepitant, showed a few translucent foci, two miliary tubercles and a grey marginal nodule with gritty foci ; lymphatic glands normal.

Strains of Virus B. IX.

Rabbits Inoculated with Culture derived from Chimpanzee 14.

The culture inoculated was the 4th generation, 22 days old ; the total age of the culture was 91 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
742	1,470	1,150	1·0 mg.	Subcutaneous	25 days (died)	In the subcutaneous tissues of the abdominal wall there was a caseous tumour the size of a pigeon's egg ; the neighbouring glands were enlarged and caseous ; there were two miliary tubercles in the lung. The pleural cavities contained a quantity of clear fluid, and the pericardial sac was distended with clear fluid containing a fibrinous clot. The left ventricle was hard, and on section showed the internal part of the wall, together with the musculi papillares, yellow and necrotic ; no tubercle bacilli were found.
743	1,270	980	0·1 mg.	Subcutaneous	103 days (died)	The local lesion was a thin-walled cavity, measuring $3 \times 2\frac{1}{4} \times 1\frac{1}{4}$, containing caseo-pus and firm caseous masses ; the nearest glands were enlarged and caseous ; the lungs did not collapse and were extensively tuberculous ; the kidneys contained sparsely scattered tubercles ; the liver and spleen were normal. The left lobe of the thyroid contained a small tubercle.
744	1,450	920	0·01 mg.	Subcutaneous	132 days (died)	The local lesion was a thin-walled cyst filled with caseo-pus ; one inguinal gland was caseous, the other neighbouring glands were normal ; the lungs contained numerous discrete caseous nodules, but were not so severely affected as usual ; the kidneys contained moderately numerous tubercles, the liver two, and the spleen none. Most of the lymphatic glands were normal. Each lobe of the thyroid contained a caseous focus.
745	1,350	920	0·001 mg.	Subcutaneous	151 days (died)	Shallow ulcer in the skin the size of a shilling ; inguinal glands enlarged and caseous throughout ; liver and spleen normal ; the kidneys contained moderately numerous tubercles ; the lungs were large and almost replaced by irregular caseous masses ; caseous masses on the diaphragm ; a nodule in each lobe of the thyroid.

Rabbits Inoculated with Culture derived from Chimpanzee 18.

The culture inoculated was the 2nd generation, 19 days old ; the total age of the culture was 36 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
993	1,540	1,150	1·0 mg.	Intravenous	16 days (died)	General tuberculosis of the usual type.
994	920	760	0·1 mg.	Intravenous	15 days (died)	
996	1,250	800	1·0 mg.	Intraperitoneal	17 days (died)	General tuberculosis.
995	900	540	0·1 mg.	Intraperitoneal	30 days (died)	General tuberculosis.
997	1,050	1,250	1·0 mg.	Subcutaneous	44 days (killed)	Local tumour the size of a hen's egg, caseo-purulent in the centre ; kidneys and spleen showed a few minute tubercles ; liver normal ; the lungs were pink and crepitant, containing a moderate number of grey tubercles with caseous centres up to the size of a millet seed.
998	840	1,150	0·1 mg.	Subcutaneous	44 days (killed)	The local lesion was a small ulcer with a caseous floor ; nearest glands caseous ; spleen contained a moderate number of yellowish tubercles the size of rape seed ; a few tubercles in each kidney ; the lungs were pink and crepitant with scattered translucent tubercles up to a millet seed.

Rabbits Inoculated with Culture derived from Monkey 110.

The Culture used was the 4th generation, 7 days ; all the generations had been grown on glycerin media ; the total age of the Culture was 55 days.

Number of Rabbit	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
521	1,380	1,010	1·0 mg.	Intraperitoneal	22 days (died)	General tuberculosis ; very numerous minute tubercles on the parietal and visceral peritoneum ; lungs beset with small tubercles ; the spleen was swollen and showed barely visible grey points ; foci not definitely visible in the liver ; many lymphatic glands showed caseous foci.
522	1,680	1,550	0·01 mg.	Intraperitoneal	37 days (died)	General tuberculosis ; the omentum was thickened and caseating; tubercles were moderately numerous on the parietal peritoneum, more numerous on the mesentery and meso-colon ; there were tubercles in all the organs, the lungs being most severely affected ; almost all the lymphatic glands were tuberculous.

Rabbits Inoculated with Culture derived from Monkey 116.

The Culture used was the 2nd generation, 18 days old ; the total age of the Culture was 27 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
691	1,220	1,030	1·0 mg.	Subcutaneous	100 days (died)	Large ulcerated caseous lesion ; adjacent glands caseous ; the lungs were enlarged, extensively consolidated, and advanced in caseation ; the kidneys contained moderately numerous nodules ; liver and spleen normal.
692	950	770	0·01 mg.	Subcutaneous	20 days (died)	Softened caseous local lesion ; inguinal glands, slightly enlarged, showed caseous foci in the cortices.
693	850	620	0·001 mg.	Subcutaneous	100 days (died)	The tuberculosis in this animal resembled that in Rabbit 691.

Rabbits Inoculated with Culture derived from Lemur 2.

The Culture used was the 6th generation, 23 days old ; the total age of the Culture was 96 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
535	1,580	1,300	1·0 mg.	Intravenous.	14 days (died)	The lungs were crepitant and contained numerous, just visible small grey tubercles with caseous, centres. The spleen was much enlarged ; (?) grey points in the pulp. Liver apparently infiltrated with extremely numerous minute grey points. No tubercles seen in the kidneys.
536	1,290	1,090	0·1 mg.	Intravenous	21 days (died)	Lungs crepitant, contained scattered miliary caseous tubercles ; spleen (greatly enlarged) and liver beset with extremely numerous minute grey points ; there were a few tubercles in the kidneys ; bronchial glands showed early caseous foci.
537	1,850	1,290	1·0 mg.	Intraperitoneal	18 days (died)	General tuberculosis of the usual type.

Rabbits Inoculated with Culture derived from Baboon 68.

The culture used was the 3rd generation, 23 days old ; the total age of the culture was 57 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
688	1,260	780	1·0 mg.	Subcutaneous	77 days (died)	Local ulcer ; adjacent glands contained caseous nodules ; lungs enlarged, extensively consolidated and caseated; there were moderately numerous nodules in the kidneys ; the spleen and liver were normal ; there were some caseous nodules in the walls of the intestines. The thoracic and abdominal (except one lumbar and one iliac) glands were normal.
689	1,560	820	0·01 mg.	Subcutaneous	129 days (died)	Small dry ulcer ; neighbouring glands caseous ; the lungs were crepitant and contained discrete caseous tubercles and nodules ; thoracic glands, liver and spleen normal ; moderately numerous caseous nodules in the kidneys ; numerous flattened caseating growths on the diaphragm and pleura and large firm nodular caseating masses in the dorsal and ventral mediastina.
690	1,470	1,280	0·0001 mg.	Subcutaneous	187 days (killed)	Small shallow ulcer ; nearest glands enlarged and caseous ; spleen and liver normal ; in the kidneys there were scattered caseous nodules up to one inch in diameter ; the lungs were closely beset with grey tubercles up to a hemp seed ; firm yellow caseous tumours in dorsal mediastinum, on pleura, pericardium, and pleural surface of the diaphragm.

Rabbits Inoculated with Culture derived from Monkey 126.

The culture was the 4th generation, 22 days ; the total age of the culture was 64 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
1016	1,000	730	1·0 mg.	Intravenous	15 days (died)	General tuberculosis ; lungs filled with small tubercles.
1015	1,300	1,050	0·1 mg.	Intravenous	19 days (died)	General tuberculosis.
1018	1,340	960	0·1 mg.	Intravenous	25 days (died)	General tuberculosis.
1014	850	—	1·0 mg.	Intraperitoneal	5 days (died)	Slight tuberculosis of omentum ; no disease elsewhere.
1017	1,070	780	0·1 mg.	Intraperitoneal	28 days (died)	General tuberculosis of the usual type.

Rabbits Inoculated with Culture derived from Monkey 132.

The culture was the 4th generation, 22 days old ; the total age of the culture was 50 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
1022	1,080	—	1·0 mg.	Intravenous	5 days (died)	No naked-eye evidence of tuberculosis.
1019	1,240	1,000	0·1 mg.	Intravenous	16 days (died)	General tuberculosis.
1020	1,440	1,150	0·1 mg.	Intravenous	21 days (died)	General tuberculosis.
1021	870	680	1·0 mg.	Intraperitoneal	23 days (died)	General tuberculosis.
1023	1,100	—	0·1 mg.	Intraperitoneal	5 days (died)	No visible disease.

RABBITS INOCULATED WITH CULTURE DERIVED FROM DOG 60.

The culture used was the 3rd generation, 21 days old, and had been in cultivation 64 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
637	2,610	1,860	0·1 mg.	Intravenous	Died, 28 days	General tuberculosis; lungs closely beset with miliary caseating coalescing tubercles.
638	2,280	1,730	0·1 mg.	Intraperitoneal	Died, 25 days	General tuberculosis; peritoneum profusely covered with small tubercles; lungs closely beset with discrete grey tubercles with caseous centres; numerous small tubercles in liver and kidneys; none visible in the spleen.
639	2,900	1,580	1·0 mg.	Intraperitoneal	Died, 28 days	General tuberculosis of the usual type.
641	1,180	900	0·1 mg.	Subcutaneous	Died, 78 days	General tuberculosis; lungs closely filled with caseating nodules; numerous tubercles in the kidneys, one minute tubercle in the spleen, none in the liver; most of the lymphatic glands were affected.
640	2,830	1,590	1·0 mg.	Subcutaneous	Died, 161 days	Softened caseous nodule the size of a hen's egg; nearest glands caseous; lungs extensively tuberculous; the kidneys contained numerous nodules; the liver a few doubtful tubercles; the spleen was normal; there were numerous tuberculous growths in the parietal pleura and the thyroid body; intestines and gall bladder were tuberculous.

RABBITS INOCULATED WITH CULTURE DERIVED FROM THE LIVER OF DOG 84.

The culture used was the 6th generation, 21 days old; the total age of the culture was 123 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
1003	1,320	950	1·0 mg.	Intravenous	Died, 17 days	Lungs packed with miliary tubercles; numerous minute foci in spleen (enlarged); liver, and kidneys.
1000	1,050	650	0·1 mg.	Intravenous	Died, 23 days	General tuberculosis; lungs packed with miliary tubercles.
1001	1,150	720	1·0 mg.	Intraperitoneal	Died, 21 days	General tuberculosis of the usual type.
1002	950	680	0·1 mg.	Intraperitoneal	Died, 32 days	General tuberculosis of the usual type.
999	850	1,250	1·0 mg.	Subcutaneous	Killed, 111 days	The local lesion consisted of a large thin-walled cyst containing a little purulent matter, contents having been evacuated through small ulcer in the skin; the nearest glands were partly caseous; the iliac glands consisted of bags of dark yellow caseo-pus; the liver and spleen were normal; the kidneys showed a few grey tubercles; the lungs collapsed, but were congested and contained a few miliary tubercles; in the margins also there were fibro-caseous nodules, one of which was filled with muco-pus. Both ankle joints and several metatarsal joints were distended with mucoid caseous substance containing numerous tubercle bacilli.

RABBITS INOCULATED WITH CULTURE DERIVED FROM THE LUNG OF DOG 50, THROUGH GUINEA-PIG 1708.

The culture used was the 3rd generation, 20 days old and had been in cultivation a total period of 75 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Result.
634	2,460	2,360	0·1 mg.	Intravenous	K. 91 days	The lungs were crepitant and contained a moderate number of flattened grey tubercles with caseous centres situated chiefly on the surface and around the margins of the lobes. Each kidney contained a few grey tubercles in the cortex, slightly opaque in the centre ; in the medullary zone of one there was a fine caseous streak. There was one minute tubercle in the heart muscle and sparsely scattered yellow tubercles in the muscle of the back (T.B. were found in the latter).
633	2,730	2,360	1·0 mg.	Intravenous	K. 91 days	The lungs and kidneys in this case were more severely affected than in Rabbit 634. The ventral portions of the cephalic lobes were composed of translucent grey tissue with an irregular caseous network, the rest of the lobes being beset with grey caseating nodules here and there confluent. In the caudal lobes there were similar nodules, which around the margins and on the dorsal surfaces had become confluent. The bronchial glands contained one or two minute yellow tubercles. The kidneys each showed on the surface a moderate number of caseous nodules, largest 2 mm., most of which extended as radial streaks into the medulla, some nearly to the apex of the papilla ; in the pelvis of one there was a small collection of caseo-pus. There were two pearly white tubercles in the liver : spleen normal.
636	1,530	930	0·1 mg.	Intraperitoneal	D. 68 days	Omentum slightly thickened and caseating ; the parietal and visceral peritoneum showed moderately numerous translucent tubercles with caseous centres. The lungs contained sparsely scattered caseating nodules, up to 3·5 mm. mainly on the surface. Other organs and glands showed no tubercles.
632	1.800	1,300	1·0 mg.	Intraperitoneal	D. 81 days	Slight tuberculosis of peritoneum. The kidneys each showed a few caseous tubercles in the cortex and caseous streaks in the pelvis. The lungs contained fairly numerous irregular caseous areas and nodules with grey margins confined mainly to the outer part and margins of the lungs. There were minute opaque foci scattered about in the muscles (T.B. numerous).
635	1,650	1,950	1·0 mg.	Subcutaneous	K. 91 days	Small flat fibrous local lesion with a small amount of caseation. Two minute foci in one inguinal gland. About 10 small caseous nodules, with grey margins, in the lungs. No tubercles elsewhere.

Strain of Virus B XXII.

RABBITS INOCULATED WITH CULTURE DERIVED FROM THE LUNG OF DOG 72.

The culture was the 2nd generation, 22 days old ; the total age of the culture was 91 days.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Method of Inoculation.	Duration of Life.	Post-mortem Results.
867	1,500	910	0·1 mg.	Intravenous	26 days (died)	General tuberculosis of the usual type.
868	1,770	1,000	1·0 mg.	Intraperitoneal	19 days (died)	General tuberculosis ; parietal visceral peritoneum closely covered with minute tubercles ; minute foci just visible in spleen (enlarged) and liver.
869	1,690	—	0·1 mg.	Intraperitoneal	8 days (died)	Coccidiosis of liver.
870	1,760	1,160	0·1 mg.	Subcutaneous	55 days (died)	Local lesion size of a hen's egg ; neighbouring glands caseous ; the lungs were crepitant and contained a moderate number of nodules up to 3 mm. ; there were a few miliary tubercles in each kidney ; the spleen and liver were normal.
871	1,660	1.000	1·0 mg.	Subcutaneous	109 days (died)	The local lesion was a shallow ulcer, $1\frac{1}{2}$ in. by $\frac{1}{2}$ in. ; nearest glands partly caseous ; spleen and liver normal ; kidneys showed sparsely scattered tubercles up to a millet-seed ; the lungs were large and extensively caseated.

INFLUENCE OF GLYCERIN ON VIRULENCE.

All the cultures used in the estimation of the virulence of different viruses for rabbits have been isolated and maintained throughout on media free from glycerin. It has been shown that when cultures of the tubercle bacillus are subcultivated on glycerin media for several generations all viruses grow with increased luxuriance, and a series of experiments on rabbits was carried out to ascertain how far this increased saprophytic power had affected the virulence.

Several viruses have been subcultivated for varying periods on glycerin media. Five of these have been tested on rabbits and the virulence of four compared with that of cultures kept on serum alone.

With each of the latter cultures, four rabbits were inoculated subcutaneously with doses of 1·0, 0·1, 0·01, 0·001 mg. respectively. Four of the rabbits died in 15, 17, 22, and 34 days respectively, and one was killed after 34 days; one of the former died of tuberculosis, the inoculation having been partly intraperitoneal; in the others the tuberculosis was limited.

Another rabbit (No. 545) lived for 315 days, gaining 770 grammes in weight, and was killed in apparent good health. The post-mortem examination showed a limited amount of general tuberculosis, of an unusually chronic type.

All the other rabbits died of general tuberculosis, the disease, as usual after subcutaneous inoculation, being most severe in the lungs and kidneys.

The results of these have been analysed and are exhibited in tabular form.

Dose.	Serum Cultures.		Glycerin Serum Cultures.	
	Number of Rabbits.	Average Duration of Life.	Number of Rabbit	Average Duration of Life.
1·0 mg.	3	96 days	4	75·75 days
0·1 mg.	4	76·5 days.	3	103·6 days.
0·01 mg.	4	114·5 days.	3	81 days.
0·001 mg.	2	80·5 days.	3	108 days.

The duration of the experiments in each series of inoculations varied very considerably, and in several instances the rabbits inoculated with the smaller doses have died before those inoculated with the larger doses, for example, in one experiment the rabbit inoculated with 0·001 mg. succumbed before any of the other three inoculated with 1·0, 0·1, and 0·01 mg.

It is clearly impossible, therefore, to draw any conclusion from a comparison of the duration of life of animals inoculated with the same dose from the different cultures.

All that the experiments can be said to show is that the glycerin cultures gave rise to a tuberculosis identical in all its features with that set up by serum cultures.

The fifth culture was inoculated intravenously into four rabbits, the same doses being employed as for the rabbits inoculated subcutaneously. In this experiment no rabbits were inoculated with the culture grown on serum alone, but a comparison of these results with those obtained with an earlier generation of the serum culture demonstrates that the culture grown on glycerin serum had not undergone any diminution in virulence.

TABLES SHOWING THE COMPARATIVE VIRULENCE OF CULTURES GROWN SINCE ISOLATION ON SERUM ALONE AND OF THE SAME CULTURE GROWN FOR A PERIOD ON GLYCERIN MEDIA.

VIRUS B. XI. (Rabbit 66).

Rabbits inoculated with culture grown since isolation on pure serum.

The subculture used was the 16th generation, 20 days old.

Rabbits inoculated with culture grown for 199 days on pure serum and 255 days on glycerin media.

The subculture used was the 20th generation, 20 days old.

The total age of the cultures in each case was 454 days. All the inoculations were made subcutaneously.

Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Duration of Life.	Result.	Number of Rabbit.	Initial Weight in Grammes.	Final Weight in Grammes.	Dose.	Duration of Life.	Result.
573	2,560	1,550	1·0 mg.	82 days (died)	Very large caseous local lesion, the skin over which was freely moveable; it infiltrated the muscles, but did not involve the peritoneum; adjacent glands enlarged and caseous; lungs enlarged and packed with caseating nodules, confluent in the cephalic lobes; the spleen and liver contained each three small tubercles; the kidneys contained numerous caseating nodules 1 to 3 mm. in diameter; the portal and thoracic glands contained small discrete tubercles; the pleura showed a few flattened caseous nodules, and there were some caseous nodules in the appendix and dilated end of ileum.	574	1,270	980	1·0 mg.	63 days (died)	Local caseous ulcer, 1 in. in diameter; neighbouring glands enlarged and caseous; the lungs filled the chest and were composed practically throughout of caseous nodules and masses; the spleen contained numerous caseous miliary tubercles; liver normal; the kidneys showed very numerous projecting caseous nodules with grey margins, up to 3 and 4 mm. in diameter; the bronchial, portal and mesenteric glands contained caseous tubercles; the appendix and dilated extremity of ileum contained numerous caseous tubercles.
564	1,150	1,000	0·1 mg.	71 days (died)	Local caseous ulcer; adjoining glands caseous; liver and spleen normal; kidneys and lungs very closely beset with caseating nodules; in the latter very little functional tissue remained; bronchial and lumbar glands slightly affected; there was one nodule on the pleura.	575	1,210	870	0·1 mg.	22 days (died)	Flattened subcutaneous caseous patch, skin adherent, but not ulcerated; inguinal glands contained caseous nodules; sternal glands a little enlarged; no tuberculosis elsewhere; liver enlarged and packed with yellow nodules (psorospermosis).
566	1,070	1,000	0·01 mg.	92 days (died)	Local ulcer; inguinal and iliac glands caseous; the lungs filled the chest and were extensively caseated; the spleen was normal; the liver contained one nodule (T.B.); the kidneys numerous nodules, up to 4 mm.; caseous foci in lumbar and bronchial glands; left lobe of thyroid enlarged and caseous throughout; caseous nodules in centre of a rib and numerous flattened nodules on the pleura.	576	1,330	1,420	0·01 mg.	81 days (died)	Caseous ulcer in abdominal wall; adjoining glands caseous; lungs enlarged and filled with caseous nodules; there were three tubercles in the spleen, two in the heart muscles, and four in the thyroid body, and numerous nodules (up to 1 cm.) in the kidneys; most of the lymphatic glands were affected.
568	1,260	1,110	0·001 mg.	64 days (died)	Large caseous local lesion infiltrating muscles of abdominal wall and involving peritoneum; skin not adherent; omentum contained a number of discrete caseous tubercles; the lungs were filled with irregular caseous nodules and masses; the kidneys contained a moderate number of caseous nodules; there was one tubercle in the spleen, and most of the abdominal and thoracic lymphatic glands were ca	577	1,070	1,150	0·001 mg.	102 days (died)	Large caseous tumour, not ulcerated; lungs and kidneys closely beset with caseating tubercles and nodules; spleen and liver normal; minute caseous tubercles in right lobe of thyroid; many lymphatic glands were tuberculous.

VIRUS B. IV. (Calf 138).

Rabbits inoculated with culture grown since isolation on pure serum.

The subculture used was the 15th generation, 21 days old.

The total age of the cultures in each case was 558 days.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.
542	1,090	870	1·0 mg.	115 days (died)	Ulcerated caseous lesion; neighbouring glands enlarged and caseous; lungs enlarged and extensively caseated; there was one tubercle in the spleen; none in the liver; the kidneys contained sparsely scattered nodules, the largest the size of a pea; there were moderately numerous miliary tubercles in the omentum; the right lobe of the thyroid was partly caseous; bronchial glands tuberculous.
543	1,400	900	0·1 mg.	94 days (died)	Large caseous lesion; skin ulcerated; nearest glands caseous; lungs firm and infiltrated with a caseous network; numerous nodules in the kidneys; spleen and liver normal; one tubercle in the thyroid body; caseous foci in mesenteric and ventral mediastinal glands; bronchial glands normal; the omentum contained a few minute tubercles.
544	1,390	1,000	0·01 mg.	103 days (died)	Local ulcer; adjoining glands caseous; lungs filled the chest and were closely beset with coalescing caseating nodules; there were three tubercles in the spleen, four in the liver and numerous projecting nodules in the kidneys; there were two tubercles in the heart muscle and a few in the omentum; most of the lymphatic glands in the body, were caseating.
545	1,280	2,050	0·001 mg.	315 days (killed)	The local lesion was an elongated fibrous tumour containing caseo-purulent nodules; yellow calcareous granules in left inguinal gland; left iliac glands formed a mass of cysts filled with caseo-pus; kidneys and liver showed a few tubercles the size of rape-seed, spleen normal; lungs a little congested and œdematous, contained scattered miliary tubercles, some caseous in the centre, and several pea-sized nodules containing caseo-pus; a lumbar and a sternal gland were enlarged and filled with caseo-pus.

Rabbits inoculated with culture grown for 302 days on pure serum and 256 days on glycerine media.

The subculture used was the 19th generation, 21 days old.

All the inoculations were made subcutaneously.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.
538	1,120	800	1·0 mg.	94 days (died)	Shallow ulcer with caseous margins; inguinal glands partly caseous; lungs crepitant, collapsed normally; they contained scattered caseous nodules and small grey tubercles with opaque centres; liver and spleen normal; the kidneys together contained ten tubercles, largest three mm. in diameter; thoracic and most of abdominal lymphatic glands were normal; there were some miliary tubercles in the omentum.
539	1,410	1,000	0·1 mg.	135 days (died)	Elongated shallow ulcer covered with dry scab; inguinal glands caseous and softened; no tubercles in spleen; a few doubtful grey points in liver; lungs enlarged, tissue extensively replaced by large caseous masses; caseous tubercle in each lobe of thyroid; caseous points in iliac, lumbar and mesenteric glands: other glands normal.
540	1,240	830	0·01 mg.	129 days. (died)	Local ulcer; nearest gland caseous; lungs collapsed moderately well and contained fairly numerous caseous nodules up to 2 or 3 mm. (in this case there was more normal lung tissue than usual); kidneys contained numerous nodules varying much in size; spleen normal; liver, a few doubtful grey points; minute tubercle in right lobe of thyroid.
541	1,200	950	0·001 mg.	114 days (died)	Local ulcer covered with dry scab; nearest glands caseous; other lymphatic glands caseous to a varying extent; lungs closely beset with more or less discrete caseous nodules (moderate amount of crepitant lung tissue); one tubercle in spleen; none in liver; numerous nodules in kidneys; numerous miliary tubercles in omentum; numerous caseous nodules in vermiform appendix and dilated end of ileum; two tubercles in a submaxillary salivary gland; large caseous nodule in one rib, expanding the bone.

VIRUS B. IV. (Calf 118).

Rabbits inoculated with culture grown on serum alone.

The subculture used was the 24th generation, 21 days old.

Rabbits inoculated with culture grown for 490 days on pure serum, and for 263 days on glycerin media.

The subculture used was the 25th generation, 21 days old, and was grown on ox serum.

The total age of the cultures in each case was 753 days. All the inoculations were made subcutaneously.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.	Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.
550	1,470	1,000	1·0 mg.	91 days (died)	Local ulcer; inguinal glands partly caseous; lungs enlarged and extensively caseated; kidneys contained numerous nodules, up to a pea in size; the spleen, two yellow tubercles; liver normal; numerous miliary tubercles in omentum, and a few flattened growths on the pleura; slight tuberculosis of thoracic and mesenteric glands.	546	1,450	960	1·0 mg.	58 days (died)	Large dense caseous tumour with a small ulcer in the skin; nearest glands caseous throughout; lungs closely beset with coalescing caseating nodules; kidneys contained numerous nodules; numerous tubercles in spleen and liver; bronchial, portal and coeliac glands partly caseous.
551	1 490	920	0·1 mg.	74 days (died)	Local ulcer; inguinal glands very large and caseous throughout; lungs contained moderately numerous caseous nodules; each kidney contained about a dozen; there were four caseous tubercles in the spleen, none in the liver; caseous tubercles in bronchial and mesenteric glands.	547	1,520	1,270	0·1 mg.	86 days (died)	Circular ulcer 1¼ inches in diameter; inguinal glands caseous throughout; lungs crepitant, contained moderately numerous discrete caseating tubercles; scattered miliary tubercles in kidneys; spleen and liver appeared normal; many lymphatic glands were caseous; three minute tubercles in the thyroid body.
552	1,840	1,150	0·01 mg.	90 days (died)	Local ulcer; inguinal glands large, caseous and softened; lungs extensively caseated; numerous caseous nodules, varying from a pin's head to a pea, in the kidneys; liver and spleen normal. Small caseous nodules in bronchial and mesenteric glands; two opaque foci in right lobe of thyroid.	548	1,830	1,230	0·01 mg.	128 days (died)	Large partially softened caseous tumour with a dry ulcer in the skin; nearest glands caseous; lungs closely beset with caseating nodules, confluent in places; the thoracic and most of the abdominal lymphatic glands were normal; spleen and liver normal; moderately numerous nodules in kidneys; left lobe of thyroid one third caseous.
553	1,440	1,450	0·001 mg.	34 days (killed)	Caseating local lesion; inguinal glands partly caseous; right caudal lobe contained one hard caseous tubercle, twice the size of a millet-seed; one tubercle in the spleen; no tubercles elsewhere.	549	1,440	1,130	0·001 mg.	34 days (died)	Flat ulcerated caseous lesion; inguinal glands partly caseous; there was a caseous focus in each iliac gland; the lungs contained two tubercles, one the size of a wheat grain, the other just visible; no tuberculosis elsewhere.

VIRUS B. XVIII. (Direct).

Rabbits inoculated with culture grown throughout on pure serum.

The subculture used was the 16th generation, 20 days old.

Rabbits inoculated with culture grown for 107 days on pure serum and for 209 days on glycerin serum.

The subculture used was the 16th generation, 20 days old, and was grown on ox serum.

The total age of the cultures in each case was 316 days. All the inoculations were made subcutaneously.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.	Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.
591	1,400	1,090	1·0 mg.	17 days (died)	Subcutaneous caseous lesion; omentum thickened and caseating; numerous minute tubercles on parietal and visceral peritoneum; sparsely scattered minute tubercles in lungs and minute grey foci in spleen and liver (inoculation partly intraperitoneal).	595	1,490	1,150	1·0 mg.	88 days (died)	Large caseous softened lesion, ulcerated on the surface; neighbouring glands tuberculous; lungs crepitant, contained numerous discrete caseating tubercles, the largest the size of a hemp-seed; moderately numerous tubercles in the kidneys; a few grey points in the liver (T.B.) and one yellow tubercle in the spleen; thoracic glands partly caseous.
589	1,270	1,100	0·1 mg.	67 days (died)	Large irregular ulcer and general tuberculosis of the usual type; both the spleen and liver contained caseous tubercles.	593	1,270	970	0·1 mg.	90 days (died)	Softened caseous tumour, with a small ulcer in the skin; neighbouring glands caseous; lungs contained a moderate number of discrete caseous nodules; the lung tissue intervening was normal in appearance; moderate number of caseous nodules in kidneys; one yellow tubercle in spleen; liver normal.
590	1,140	1,250	0·01 mg.	173 days (died)	Very large cyst, with caseo-purulent contents in subcutaneous tissues of abdominal wall; neighbouring glands enlarged and caseous; lungs closely beset with coalescing caseating masses; kidneys packed with caseous nodules; liver and spleen appeared normal; omentum retracted and beset with caseating tubercles; tendon of diaphragm covered with firm fibro-caseous masses, 1 cm. in greatest thickness; large caseating masses in dorsal and ventral mediastina, latter completely covering costal cartilages; numerous filmy growths on parietal pleura; most of the lymphatic glands were caseous.	594	1,170	1,140	0·01 mg.	86 days (died)	Large softened caseous tumour, ulcerated on the surface; inguinal glands enlarged and caseous; lungs packed with caseating nodules; sparsely scattered nodules, mostly small, in kidneys; spleen enlarged, Malphigian bodies prominent; liver normal; opaque tubercle in thyroid body (T.B.).
592	1,100	1,150	0·001 mg.	97 days (died)	Large caseous ulcerated lesion and general tuberculosis of the usual type, the lungs being the most severely affected; the liver contained psorosperm nodules, but no tubercles.	596	1,010	680	0·001 mg.	15 days (died)	Small caseous ulcer; nearest glands normal; there was one translucent tubercle in the lungs.

Virus B. V. (G.P. 711).

RABBITS INOCULATED WITH CULTURE GROWN FOR 743 DAYS ON PURE SERUM AND 166 DAYS ON GLYCERIN MEDIA.

The subculture used was the 21st generation, 21 days old, and was grown on ox serum.

The total age of the culture was 809 days.

All the inoculations were made intravenously.

Number of Rabbit.	Initial weight in grammes.	Final weight in grammes.	Dose.	Duration of Life.	Result.
790	1,300	950	1·0 mg.	15 days (died)	Lungs collapsed fairly well and contained numerous discrete grey tubercles, the largest the size of a millet seed ; the bronchial glands showed opaque caseous foci in the cortex ; spleen enlarged, firm, peppered with minute grey foci ; there were a few grey points just visible in the liver and one or two minute foci in each kidney ; a few opaque foci in cortex of portal and lumbar glands.
791	1,550	910	0·1 mg.	21 days (died)	Lungs crepitant but a little fuller than normal ; they contained fairly numerous miliary caseating tubercles ; the bronchial glands contained caseous nodules ; spleen enlarged, firm and dark red, closely beset with minute yellowish foci ; liver speckled throughout with minute grey foci ; moderately numerous caseating tubercles in the kidneys ; minute caseous tubercles in most of the lymphatic glands.
792	1,750	1,320	0·01 mg.	62 days (died)	The lungs were enlarged, firm, and composed for the most part of grey translucent tissue with an irregular caseous network. Spleen slightly enlarged, contained moderately numerous discrete yellow tubercles ; in the cortex of each kidney were numerous grey tubercles, with caseous centres, many of which extended towards the pelvis as radial streaks ; liver appeared normal ; cortex of portal gland partly caseous ; most other lymphatic glands contained caseous tubercles ; scattered about the muscles were moderately numerous yellow tubercles up to a pin head in size.
793	1,200	1,000	0·001 mg.	52 days (died)	Lungs were pinkish and crepitant and contained sparsely scattered caseous tubercles, up to 2 mm. in diameter, with grey margins ; they were irregular in outline and situated chiefly just under the pleura ; the bronchial glands showed a few opaque foci ; the mediastinal were partly caseous ; there was some turbid fluid in the peritoneal cavity ; spleen enlarged, firm and closely beset with irregular greyish white foci, here and there aggregated together ; the liver showed numerous minute grey foci and grey submiliary tubercles; kidneys appeared normal ; several lymphatic glands contained caseous tubercles.

REPORT ON THE STABILITY OF VIRULENCE OF THE TUBERCLE BACILLUS OF HUMAN ORIGIN IN ARTIFICIAL CULTURE, INCLUDING THE INFLUENCE OF GLYCERIN IN CULTURE MEDIA.

Dr. L. COBBETT.

THE STABILITY OF VIRULENCE OF THE TUBERCLE BACILLUS IN ARTIFICIAL CULTURE.

The Virulence of Tubercle Bacilli from Cultures and in Emulsions of Tuberculous Animal Tissues compared.

It has been repeatedly observed, both here and at Walpole, that to produce tuberculosis of a given severity in an animal a much larger number of tubercle bacilli are required when these are obtained from a culture, even a young one, than when they are contained in an emulsion of tuberculous tissue from an infected animal. Dose for dose, estimated in numbers of tubercle bacilli, cultures are decidedly less virulent than emulsions of tuberculous tissue*. It is difficult to say whether this is because cultures contain a larger proportion of dead bacilli, whether tuberculous tissues contain poisons which cultures do not, whether the tissue matter acts mechanically and protects the bacilli from the attack of the defensive forces of the body, or whether an attenuation of the bacilli takes place when they are transferred from their natural habitat to our artificial culture media.

If the last of these be the true explanation, then one would expect the attenuation to be not altogether sudden and complete, but to extend over the earlier period of cultivation for perhaps several weeks, so that it should be possible to demonstrate the attenuation by comparing the virulence of very early cultures with others which had been growing for a longer period.

No direct experiments of this kind have been made, but seeing that our viruses of Group I. differ in virulence from one another so little that the difference has not been demonstrated by our experiments (H 49, T.C. excepted), it is possible to compare the virulence of recently isolated strains of culture of one virus with those of another which have been longer in cultivation. For this purpose several strains have been raised as quickly as possible, and bacilli from cultures three weeks old have been injected within 5, 7, or 8 weeks of the time when the virus was first sown on artificial media. These can be compared with other strains which have been several years under artificial cultivation.

This comparison is, of course, not an exact one, for not only were different viruses employed, but the calves were not of precisely the same size, and differed much. as we know, in individual susceptibility. Yet the results are very fairly uniform, and had any considerable attenuation taken place during the period of cultivation it could not have failed to make itself known.

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* Experience here has shown that to produce a rapidly fatal tuberculosis in the calf by subcutaneous injection about 500,000,000 tubercle bacilli direct from an animal lesion are required, while, if cultures be used, 50 mg. is necessary, 10 mg. being not certainly fatal. (Compare the table of tissue emulsion injections with that of culture injections; Vol. II., Section II.) Since Dr. Eastwood estimates that 10 mg. contains 45,000,000,000 bacilli, the bacilli from recent tuberculous lesions in the animal (guinea-pig, calf, or rabbit) would appear to be equivalent to more than 100 times as many obtained from a young serum culture.

Table showing the Relation of the Total Age of Culture (time since isolation from animal during which the bacilli have grown in artificial cultivation on pure serum) to Virulence.

Group I. Viruses.

All Cultures injected were grown for 3 weeks.

Dose: 50 Milligrammes.

Virus.	Direct Source of Culture.	No. of Calf.	Total Duration of Artificial Cultivation.	Duration of Life of Calves.	
H 31. “L.F.”	Human cervical glands	**519**	5 weeks	Died	21 days
	,, ,	**521** (30 mg)	,,	Dying	36 ,,
H 59. “L.B.”	Human lung	**965**	7 weeks	,,	60 ,,
	Human mesenteric glands, through G.P.1849.	**1063**	2 months	Died	28 ,,
H 64. “M.G.”	Human meninges, through G.P. 1880.	**1091**	,,	Dying	37 ,,
	Human mesenteric glands, through G.P. 1910.	**1115**	,,	Died	38 ,,
H 49. “T.C.”*	Calf 797	**931**	,,	,,	21 ,,
	,,	**949**	,,	,,	39 ,,
H 32. “Y.W.”	Human mesenteric glands	**587**	3 months	,,	35 ,,
	,, ,,	**589**	,,	,,	40 ,,
H 16. “J.H.”*	Bull 423A (5th passage)	**541**	,,	Very ill	25 ,,
	,, ,,	**559**	,,	Dying	39 ,,
H 17. “Sp.B.”*	Calf 571 (7th passage)	**739**	3½ months	,,	33 ,,
	,, ,,	**743**	,,	,,	37 ,,
H 65. “K.B.”	Human mesenteric glands, through G.P. 1835.	**1093**	3⅔ months	Died	30 ,,
H 28. “C.L.”	Human cervical glands	**489**	4 months	Dying	28 ,,
	,, ,,	**469** (43 mg)	,,	Very ill	29 ,,
H 32. “Y.W.”	Human bronchial glands, through Rabbit 166.	**837**	6 months	Died	33 ,,
	,, ,, ,,	**849**	,,	,,	23 ,,
H 29. “M.F.”	Human cervical glands, through G.P. 1329.	**693**	8 months	,,	63 ,,
	,, ,, ,,	**697**	,,	Dying	35 ,,
H 10. “B.S.”	Calf 113	**387**	,,	,,	34 ,,
H 20. “F.L.”	Calf 213	**547**	,,	Died	36 ,,
	,,	**561**	,,	,,	33 ,,
H 19. “S.W.”	Heifer 239	**577**	10 months	,,	37 ,,
	,,	**597**	,,	Dying	45 ,,
H 29. “M.F.”	Human cervical glands, through G.P. 1329.	**841**	11 months	Died	23 ,,
	,, ,, ,,	**847**	,,	Very ill	31 ,,
H 38. “J.M.”	Human cervical glands, through G.P. 793.	**643**	14 months	Died	28 ,,
	,, ,, ,,	**647**	,,	,,	32 ,,
H 13. “A.D.”*	Calf 321	**825**	15½ months	,,	25 ,,
H 10. “B.S.”	Calf 113	**667**	16½ months	Well	90 ,,
H 14. “F.S.”	Human mesenteric glands, through G.P. 587.	**545**	16 months	Died	45 ,,
	,, ,, ,,	**671**	19 months	,,	33 ,,
H 2. “Sp.A.”	Heifer 11	**569**	20 months	Dying	20 ,,
	,,	**575**	,,	,,	33 ,,
H 7. “C.M.”	Calf 5, through G.P. 448	**563**	20 months	Very ill	32 ,,
	,, ,,	**773**	25 months	Dying	40 ,,
	,, ,,	**775**	,,	,,	47 ,,
H 14. “F.S.”	Human mesenteric glands, through G.P. 587.	**899**	2 yrs., 3 months	Died	44 ,,

* After increase of virulence.

Does Long-continued Cultivation on Artificial Media Attenuate the Tubercle Bacillus?

The evidence of attenuation during artificial cultivation afforded by the calves injected with 50 mg. of bacilli is of the slightest (*see* Table, page 188). The youngest strain, which had only been five weeks under cultivation when injected, killed a calf in almost the shortest period, namely, 21 days. One two months old killed as quickly. But another, cultivated for 20 months, killed even more rapidly, namely, in 20 days. One calf alone survived the period of observation. It had been injected with a strain which had been cultivated for 16½ months. Possibly all these were exceptional animals.

Twenty-eight calves were injected with bacilli from strains which had been less than a year in cultivation; all died or were in a dying condition when killed. Twelve were injected with bacilli from strains more than a year in cultivation; all died or were killed when dying, except the one which was in good condition when killed 90 days after injection. Counting the duration of life of this one as 90 days, the average duration of illness for the two groups works out at 34·6 and 39 days respectively. Strains which had been more than two years in cultivation killed in 40, 44, and 47 days.

Thus the evidence of this table is not opposed to a slight and slowly progressing attenuation.

Table showing the Relation of the Total Age of Culture (time since isolation from animal during which the bacilli have grown in artificial cultivation on pure serum) to Virulence.

Group I. Viruses.

All Cultures injected were grown for 3 weeks.

Dose: 10 Milligrammes.

Virus.	Direct Source of Culture.	No. of Calf.	Total Duration of Artificial Cultivation.	Duration of Life of Calves.	
H 59. "L.B."	Human lung	**987**	7 weeks	Dying	49 days
	,, ,,	**991**	,,	Well	94 ,,
	Human cervical glands	**1037**	2 months	Died	41 ,,
	Human brain, through G.P. 1860	**1057**	,,	,,	31 ,,
	Human mesenteric glands, through G.P. 1849.	**1077**	,,	,,	31 ,,
H 64. "M.G."	Human mesenteric glands, through G.P. 1910.	**1111**	,,	Dying	60 ,,
	,, ,, ,,	**1113**	,,	Well	86 ,,
H 49. "T.C."*	Calf 797	**933**	,,	Died	19 ,,
	,,	**929**	,,	Dying	39 ,,
H 17. "Sp. B."*	Calf 553 (5th Passage)	**901**	3 months	Died	54 ,,
	,, ,,	**919**	,,	,,	31 ,,
H 65. "K.B."	Human mesenteric glands, through G.P. 1835.	**1085**	3⅔ months	Dying	51 ,,
	,, ,, ,,	**1089**	,,	Died	38 ,,
H 32. "Y.W."	Human bronchial glands, through Rabbit 166.	**851**	6 months	,,	47 ,,
	,, ,, ,,	**855**	,,	,,	34 ,,
H 29. "M.F."	Human cervical glands, through G.P. 1329.	**835**	11 months	,,	40 ,,
	,, ,, ,,	**839**	,,	Dying	69 ,,
H 28. "C.L."	Human cervical glands	**813**	13 months	Died	39 ,,
	,, ,,	**815**	,,	Dying	48 ,,
	Human cervical glands, through Rabbit 102.	**863**	14½ months	Well	90 ,,
	,, ,, ,,	**877**	,,	Died	37 ,,
	,, ,, ,,	**819**	,,	,,	52 ,,
	,, ,, ,,	**875**	,,	Well	91 ,,
H 31. "L.F."	Human cervical glands	**939**	14 months	Died	43 ,,
	,, ,,	**947**	,,	,,	72 ,,
H 13. "A.D."*	Calf 321	**821**	15½ months	Dying	50 ,,
	,,	**823**	,,	Well	90 ,,
H 20. "F.L."	Calf 213	**879**	17 months	Dying	36 ,,
	,,	**867**	,,	Well	90 ,,
H 19. "S.W."	Goat 11	**921**	18 months	Died	48 ,,
	,,	**913**	,,	Well	90 ,,
H 38. "J.M."	Human cervical glands, through G.P. 793.	**845**	19½ months	Dying	36 ,,
	,, ,, ,,	**827**	,,	Well	108 ,,
H 2. "Sp. A."	Calf 93, through G.P. 534	**889**	2 yrs. 3 mos.	Died	53 ,,
	,, ,,	**883**	,,	Well	90 ,,
H 14. "F.S."	Human mesenteric glands, through G.P. 587.	**893**	,,	Died	47 ,,
	,, ,, ,,	**895**	,,	In fairly good health	90 ,,
H 10. "B.S."	Calf 35, through G.P. 725	**967**	,,	Dying	48 ,,
	,, ,,	**969**	,,	,,	48 ,,
H 7. "C.M."	Calf 5, through G.P. 448	**1029**	3 years	Well	90 ,,
	,, ,,	**1041**	,,	Died	41 ,,

* After increase of virulence.

Among the calves injected with 10 mg. of bacilli about 1 in 4 survived for 90 days, and many of these were in good condition and had only a slight amount of tuberculosis when killed. It is perhaps significant that 9 of these less severely affected animals occurred among the 24 calves injected with strains which had been cultivated for more than a year, and two only among the 17 injected with strains which had been cultivated for less than a year.

But these calves were highly-resisting individuals, for their fellows, treated like themselvs, died of acute tuberculosis; it would not be reasonable to expect the exceptional individuals to be distributed equally among two such groups as we are considering. It may therefore be that a capricious distribution of animals of high resistance accounts for this evidence of attenuation.

If we leave out all the calves which survived 90 days, regarding them as exceptionally resistant individuals, and consider only those which died—though doubtless these also differed from one another in susceptibility—we find that of 15 animals injected with strains which had been less, and 15 with strains over a year in artificial cultivation, the average duration of illness was 42·3 and 46·5 days respectively. Here again the evidence is in favour of a slowly progressing attenuation.

ANALYSIS OF THE TABLE OF RABBIT INJECTIONS.

Arranged in the order of the duration of cultivation of the viruses.

Virus.	Strain of Culture used for Rabbit injections.	Total Duration of Artificial Cultivation.	Dose in Milligrammes.			
			10·	1·	0·1	0·01
H 31. "L.F."	Human cervical glands	5 weeks	–	–	47	–
H 59. "L.B."	Human lung	7 weeks	–	22	?	–
	Human cervical glands	2 months	–	18	69	–
	Human brain	"	–	23	?	–
	Human mesenteric glands	"	–	34	34	–
H 64. "M.G."	" " "	"	–	25–21	34	–
H 49. "T.C."*	Calf 797	"	15	21–21	24	–
H 14. "F.S."	Calf 895	2½ months	–	24	33	–
H 32. "Y.W."	Human mesenteric gland	3 months	–	21	30	–
H 16. "J.H."*	Bull 423A	"	23	28	60	–
H 17. "Sp.B."*	Calf 571	3½ months	16	23	37	40
H 65. "K.B."	Human mesenteric gland	3⅔ months	17	28–30	28	–
H 32. "Y.W."	Human bronchial gland	6 months	32	?	15	27
H 29. "M.F."	Human cervical gland	7 months	16	18	22	29
	" " "	8 months	–	22	30	91
H 20. "F.L."	Calf 213	"	17	18	30	–
H 19. "S.W."	Heifer 239	10 months	20	19	42	32
H 29. "M.F."	Human cervical glands	11 months	11	15	?	?
H 2. "Sp.A."	Calf 91	13 months	25	39	35	–
H 28. "C.L."	Human cervical glands	"	36	40	40	?
H 38. "J.M."	" " "	14 months	15	22	–	–
H 31. "L.F."	" " "	"	25	31–26	–	–
H 28. "C.L."	Rabbit 102	14½ months	16	23	23	31
H 13. "A.D."*	Calf 321	15½ months	?	33-36	?	–
H 10. "B.S."	Calf 113	16½ months	21	23	44	78
H 14. "F.S."	Human mesenteric glands	16 months	21	29	57	–
H 20. "F.L."	Calf 213	17 months	18	25–19	25	–
H 19. "S.W."	Goat 11	18 months	9	17	24	–
H 2. "Sp.A."	Heifer 11	"	17	35	24	–
H 14. "F.S."	Human mesenteric glands	19 months	18	23	23	29
H 38. "J.M."	Human cervical glands	19½ months	10	17	19	23
H 2. "Sp.A."	Heifer 11	20 months	17	23	35	–
H 7. "C.M."	Calf 5	"	22	27	55	–
	"	25 months	16	37	?	39
H 14. "F.S."	Human mesenteric glands	27 months	–	?	25–23	–
H 2. "Sp.A."	Calf 93	"	17	31–42	34	–

* After increase of virulence.

The analysis of the rabbit injections gives no evidence of attenuation during cultivation.

SUMMARY.

Both tables of calf injections agree together in showing that strains of bacilli which have been long in cultivation are slightly less virulent than those which have been more recently isolated. The number of animals is probably too great to admit of this agreement being merely accidental. We must, therefore, conclude that a slowly progressing attenuation does take place during long continued cultivation. But the striking fact shown by these tables is not the evidence of attenuation, but on the contrary, the slightness of this evidence, which strongly testifies to the remarkable stability of virulence [as compared with some other micro-organisms] of the tubercle bacillus, which hardly becomes appreciably attenuated after several years cultivation. There is no evidence that attenuation is more rapid in the early days of cultivation than it is at a later period. The analysis, therefore, lends no support to the view that bacilli from cultures are *intrinsically* less virulent than those from tissue emulsions.

THE INFLUENCE OF GLYCERIN IN CULTURE MEDIA.

All the experiments already considered in this Report were made with cultures on pure serum. It has been suggested that glycerin in culture media may attenuate tubercle bacilli. This is of importance because some of our Group II. viruses have been injected after growing on glycerin-serum, and this medium ha sbeen largely used by me for separating mixtures of eugonic and dysgonic tubercle bacilli, or for attempting such separation from strains which came under suspicion. It was there desirable to investigate whether glycerin in culture media has any attenuating action

Two questions presented themselves—

(1) If two cultures be sown, from a stock hitherto grown on pure serum, on serum with and on serum without glycerin, will there be any difference in the severity of the illness caused by injecting them into animals in equal quantities ?

(2) Does long-continued cultivation on glycerin-media attenuate ?

In order to answer these questions many animals were injected in pairs, one of each pair with a culture grown on serum and the other with one grown on 5 per cent. glycerin-serum

Calf Injections.

In the case of only one virus of Group I., H 7, "C.M.," have comparative injections been made on calves. In that case there was no appreciable difference in the result. Four non-virulent strains were injected, for the purpose, as already described, of controlling those calf injections which have already been made with non-virulent cultures. They show no appreciable difference in the result, whether cultures on pure serum or on serum with 5 per cent. of glycerin were used.

Rabbit Injections.

A much larger number of comparative injections with viruses of Group II. have been made on rabbits. These also show no appreciable difference in pathogenicity between cultures grown on pure serum and those on glycerin-serum. For the sake of brevity the table is omitted.

Comparative injections have been made on rabbits with eleven strains (ten viruses) of bacilli of Group I.

In some cases where life was much prolonged the cause has been found in the fact that the injection was not really intraperitoneal, but only partly intraperitoneal and partly intramuscular, or intracœcal. Omitting these spoilt experiments, there is a clear consensus of evidence that pure serum cultures kill quicker than those on glycerin-serum. The difference is not great, but is fairly constant (with a few exceptions, easily explicable on the hypothesis of variations in individual susceptibility).

TABLE SHOWING COMPARATIVE SUBCUTANEOUS INOCULATIONS OF SERUM AND GLYCERIN-SERUM CULTURES OF VARIOUS VIRUSES INTO CALVES.

A. SERUM CULTURES.

CALVES.

Virus.	Immediate source of Culture.	Dose in Milli-grammes.	No. of Calf.	Weight in Kilogrms.	Result.		
	VIRUSES OF GROUP I.						
H 7. "C.M." - -	G.P. 448, from Calf 5.	50·	**773**	50·34	Killed, when dying,	after 40 days	General tuberculosis, severe.
	VIRUSES OF GROUP II.						
H 44. "D.C." -	Human cervical glands, direct.	50·	**731**	46·70	Killed, when well,	after 90 days	Slight tuberculosis.
H 18. "T.T." - -	Calf 405.	50·	**645**	50·80	„	„ 90 „	Slight tuberculosis.
H 30. "E.M." - -	G.P. 1337, from human bronchial and mesenteric glands.	50·	**593**	69·84	„	„ 88 „	Slight tuberculosis.
H 11. "E.D." - -	G.P. 1298, from Calf 309.	50·	**719**	49·40	„	„ 90 „	Local tuberculosis.

TABLE SHOWING COMPARATIVE SUBCUTANEOUS INOCULATIONS OF SERUM AND GLYCERIN SERUM CULTURES OF VARIOUS VIRUSES INTO CALVES.

B.—GLYCERIN SERUM CULTURES.

CALVES.

Virus.	Immediate source of Culture.	Dose in milli-grammes.	No. of Calf.	Weight in Kilogrms.	Result.		
	VIRUSES OF GROUP I.						
H 7. "C.M."	G.P. 448, from Calf 5.	50·	**775**	·70	Killed when dying,	after 47 days	General tuberculosis, severe.
	VIRUSES OF GROUP II.						
H 44. "D.C."	Human cervical gland, direct.	50·	**705**	52·16	Killed when well,	after 90 days	Slight tuberculosis.
H 18. "T.T."	Calf 405.	50·	**683**	58·06	,, ,,	,, 90 ,,	Local tuberculosis.
H 30. "E.M."	G.P. 1337 from human bronchial and mesenteric glands.	50·	**599**	63·95	,, ,,	,, 88 ,,	Local tuberculosis.
H 11. "E.D."	G.P. 1298 from Calf, 309.	50·	**723**	44·90	,, ,,	,, 90 ,,	Slight tuberculosis.

TABLE SHOWING COMPARATIVE INTRAPERITONEAL INOCULATIONS OF PURE SERUM AND GLYCERIN SERUM CULTURES OF VARIOUS VIRUSES INTO RABBITS.

A. SERUM CULTURES—Viruses of Group I.

RABBITS.

Virus.	Immediate Source of Culture.	Dose in Milligrammes	No. of Rabbit.	Weight in grms.	Killed or Died.	Duration of Life in Days	Results.
H 29. "M.F."	G.P. 1329 from human cervical glands.	1·	**315**	1,020	Died	22	General tuberculosis.
	,,	0·1	**316**	1,000	,,	30	,, ,,
	,,	0·01	**317**	840	,,	91	,, ,,
H 7. "C.M."	G.P. 448 from Calf 5	10·	**408**	1,100	Died	16	General tuberculosis.
	,,	1·	**409**	1,200	,,	37	,, ,,
	,,	0·1	**410**	1,020	Killed	94	Chronic tuberculosis of lungs and kidneys. (Injection intramuscular and intracœcal.)
	,,	0·01	**411**	1,130	Died	39	General tuberculosis.
H 10. "B.S."	G.P. 757 from Calf 113	10·	**298**	1,390	Died	21	General tuberculosis.
	,,	1·	**297**	1,390	,,	23	,, ,,
	,,	0·1	**296**	1,360	,,	44	,, ,,
H 38. "J.M."	G.P. 793 from human cervical glands.	10·	**488**	1,120	Died	10	General tuberculosis.
	,,	1·	**489**	1,010	,,	17	,, ,,
	,,	0·1	**490**	920	,,	19	,, ,,
	,,	0·01	**491**	1,200	,,	2	,, ,,

Table Showing Comparative Intraperitoneal Inoculations of pure Serum and Glycerin-Serum Cultures of Various Viruses into Rabbits.

B. GLYCERIN-SERUM CULTURES—Viruses of Group I.

RABBITS.

Virus.	Immediate Source of Culture.	Dose in Milligrammes	No. of Rabbit.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H. 29. "M.F." -	G.P. 1329 from human cervical glands.	1·	**312**	1,260	Died	73	General tuberculosis of a chronic type.
	"	0·1	**313**	860	"	41	General tuberculosis.
	"	0·01	**314**	670	"	107	" "
H. 7. "C.M." -	G.P. 448 from Calf 5.	10·	**404**	1,850	Died	25	General tuberculosis.
	"	1·	**405**	1,300	"	7	" "
	"	0·1	**406**	1,150	Killed	94	Chronic tuberculosis, rather severe in lungs. (Injection intramuscular and intracœcal).
	"	0·01	**407**	1,150	Died	94	General tuberculosis of a chronic type.
H. 10. "B S." -	G.P. 757 from Calf 113	10·	**401**	1,000	Died	17	General tuberculosis.
	"	1·	**402**	1,020	"	39	" "
	"	0·1	**403**	1,540	"	26	" "
H. 38. "J.M." -	G.P. 793 from human cervical glands.	10·	**492**	1,080	Died	22	General tuberculosis. There seemed hardly enough tuberculosis to account for death.
	"	1·	**493**	1,150	"	32	General tuberculosis.
	"	0·1	**494**	1,230	"	52	" "
	"	0·01	**495**	970	Killed (when very ill).	78	General tuberculosis, not severe.
	3rd Culture on Glycerin Serum.						
do. do.	G.P. 793 from O.M.	10·	**549**	1,320	Died	26	General tuberculosis, severe acute type.
	"	10·	**550**	1,020	"	25	General tuberculosis.
	"	1·	**551**	1,170	"	32	" "
	"	1·	**552**	950	"	29	" "

A. SERUM CULTURES—Viruses of Group I.

RABBITS—*continued.*

Virus.	Immediate Source of Culture.	Dose in Milli-grammes	No. of Rabbit.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H. 32. "Y.W." -	Rabbit 166 from human bronchial glands.	10·	**497**	1,050	Died	32	General tuberculosis. Injection partly intracœcal.
	,,	1·	**498**	1,220	,,	12	Early general tuberculosis, probably accelerated by psorospermosis.
	,,	0·1	**499**	1,200	,,	15	General tuberculosis.
	,,	0·01	**500**	1,050	,,	27	,, ,,
H. 20. "F.L" -	Calf 213, direct.	10·	**571**	1,810	Died	18	General tuberculosis.
	,,	1·	**572**	1,430	,,	25	,, ,,
	,,	1·	**573**	1,480	,,	19	,, ,,
	,,	0·1	**574**	1,220	,,	25	,, ,,
H. 19. "S.W." -	Goat 11, direct.	10·	**607**	1,120	Died	9	Tuberculous peritonitis.
	,,	1·	**608**	820	,,	17	General tuberculosis.
	,,	1·	**609**	920	,,	6	Death probably from cysticercosis.
	,,	0·1	**610**	870	,,	24	General tuberculosis.
H. 14. "F.S." -	GP. 587, from human mesenteric glands.	1·	**619**	690	Died	8	Early tuberculosis; ? cause of death.
	,,	1·	**621**	940	,,	14	General tuberculosis.
	,,	0·1	**620**	970	,,	25	General tuberculosis, complicated by cysticercosis.
	,,	0·1	**622**	670	,,	23	General tuberculosis.
H. 17. "Sp. B." (after increase of virulence.)	GP. 1542, from Calf 553.	10·	**599**	2,830	Died	13	Early general tuberculosis; ? cause of death.
	,,	1·	**600**	1,560	,,	19	General tuberculosis.
	,,	1·	**601**	1,310	,,	81	General tuberculosis. (Injection partly intramuscular.)
	,,	0·1	**602**	1,360	,,	119	General tuberculosis. (Injection intracœcal).
H. 16. "J.H." - (after increase of virulence).	Calf 337, direct.	10·	**445**	1,120	Died	11	General tuberculosis.
	,,	1·	**446**	1,020	,,	18	Early tuberculosis. Death perhaps accelerated by psorospermosis.
	,,	0·1	**447**	1,370	,,	28	General tuberculosis.
do. do.	Calf 317 direct.	1·	**356**	1,020	Died	24	General tuberculosis.
	,,	0·1	**357**	810	,,	26	,, ,,

B. GLYCERIN-SERUM CULTURES—Viruses of Group I.

RABBITS—*continued.*

Virus.	Immediate Source of Culture.	Dose in Milligrammes	No. of Rabbit.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H 32. "Y.W." -	Rabbit 166, from human bronchial glands.	10·	**501**	1,000	Died	14	Early tuberculosis. Death accelerated by psorospermosis. (Injection partly subcutaneous.)
	,,	1·	**502**	1,400	,,	14	General tuberculosis.
	,,	0·1	**503**	1,250		26	,, ,,
	,,	0·01	**504**	1,180	,,	36	,, ,,
H 20. "F.L." - -	Calf 213, direct.	10·	**575**	1,420	Died	16	General tuberculosis.
	,,	1·	**576**	1,450	,,	63	General tuberculosis. (Injection partly intramuscular.)
	,,	1·	**577**	1.400	,,	25	General tuberculosis.
	,,	0·1	**578**	1,280	,,	34	,, ,,
H 19. "S.W." -	Goat 11, direct.	10·	**611**	900	Died	31	General tuberculosis, complicated by cysticercosis.
	,,	1·	**612**	900	,,	30	Tuberculosis. Death due to other causes.
	,,	1·	**613**	1,040	,,	76	Chronic tuberculosis of peritoneum and lungs. Kidneys affected slightly. Severe cysticercosis.
	,,	0·1	**614**	960	Killed	90	Chronic general tuberculosis. (Injection partly intracoecal.)
H 14. "F.S." -	G. P. 587, from human mesenteric glands.	1·	**623**	1,040	Died	16	General tuberculosis, early.
	,,	1·	**625**	890	,,	15	General tuberculosis.
	,,	0·1	**624**	920	,,	20	,, ,,
	,,	0·1	**626**	800	,,	35	,, ,,
H17. "Sp.B." (after increase of virulence).	G. P. 1542, from Calf 553.	10·	**603**	1,250	Died	15	General tuberculosis, complicated by cysticercosis.
	,,	1·	**604**	1,190	,,	22	General tuberculosis.
	,,	1·	**605**	1,150	,,	18	,, ,,
	,,	0·1	**606**	1,070	,,	59	,, ,,
H 16. "J.H." (after increase of virulence).	Calf 337, direct.	10·	**442**	1,020	Died	13	,, ,,
	,,	1·	**443**	1,420	,,	24	,, ,,
	,,	0·1	**444**	850	,,	38	,, ,,
do. do.	Calf 317, direct.	1·	**358**	1,140	Died	19	,, ,,
	,,	0·1	**359**	1,120	,,	45	,, ,,

ANALYSIS OF THE PRECEDING TABLE,

Showing the comparative virulence for the rabbit (indicated by the duration of life) of pure serum and glycerin-serum cultures.

Dose in milli-grammes.	H 29. "M.F."		H 7. "C.M."		H 10. "B.S."		H 38. "J.M."		H 32. "Y.W."		H 20. "F.L."	
	S.	G.S.	S.	G. S.	S.	G S.	S.	G.S.	S.	G.S.	S.	G.S.
10·	–	–	16	25	21	17	10	22	–	–	18	16
1·	22	?73	37	37	23	39	17	32	–	14	25–19	25
0·1	30	41	–	–	44	26	19	52	15	26	25	34
0·01	91	107	39	94	–	–	23	78	27	36	–	–

	H 19. "S.W."		H 14. "F.S."		H 17. "Sp.B."† [5th Passage.]		H 16. "J.H."† [3rd Passage.]		H 16. "J.H."† [4th Passage.]		AVERAGE.	
	S.	G.S.	S.	G.S.	S.	G.S.	S.	G.S.	S.	G.S.	S.	G.S.
10·	9	31			13	15	11	13		–	**14**	**20**
1·	17	76	14	16–15	19	22–18		24	24	19	**21·7**	**28**
0·1	24	–	23	20–35	–	59	28	38	26	45	**26·**	**37·6**
0·01		–		–	–	–	–	–	–	–	**45**	**79**

S. = Pure serum cultures. G.S. = Cultures on serum + 5 per cent. of glycerin. † After increase of virulence.

The numbers in the columns headed "S" represent the duration of life in days of rabbits injected with different doses of serum cultures; the opposite numbers in the columns headed "G.S." represents the duration of life of rabbits injected with similar doses of glycerin-serum cultures. The average duration of life of the rabbits inoculated with serum and glycerin-serum cultures respectively in each of the four doses used is also shown.

All those animals in which the tuberculosis was seriously complicated by other disease, or in which there was evidence that the injection had not been entirely intraperitonal, are omitted. This accounts for the gaps in the analysis.

The difference between the virulence of the serum and the glycerin-serum cultures is greater with some viruses than with others. Thus, it is great in the case of H 38 "J.M.," and H 19 "S.W.," which are less dysgonic, while it is least in the very dysgonic viruses, H 10 "B.S.," H 14 "F.S.," and H 16 "J.H." (fourth passage).

GUINEA-PIG INJECTIONS.

Comparative injections with cultures on glycerin-serum and pure serum have been made upon guinea-pigs also. Three viruses of Group I. and three of Group II. were employed for this purpose. The results are given in the following tables.

TABLE SHOWING COMPARATIVE INTRAPERITONEAL INOCULATIONS OF PURE SERUM AND GLYCERIN-SERUM CULTURES OF VARIOUS VIRUSES INTO GUINEA-PIGS.

A. SERUM CULTURES—Viruses virulent for bovines.

GUINEA-PIGS.

Virus.	Immediate Source of Culture.	Dose in Milli-grammes	No. of G.P.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H 38. "J.M" -	G.P. 793, from human cervical glands.	1·0	**580**	440	Died	14	General tuberculosis.
	,,	1·0	**581**	520	,,	16	General tuberculosis. Death probably hastened by other causes.
	,,	0·1	**582**	470	,,	21	General tuberculosis.
	,,	0·1	**583**	500	,,	21	,, ,,
H 32. "Y.W." -	Rabbit 166, from human bronchial glands.	1·0	**590**	250	Died	18	General tuberculosis. (Injection partly intracœcal.)
	,,	1·0	**591**	230	,,	16	General tuberculosis.
	,,	0·1	**592**	280	,,	16	General tuberculosis, and septic peritonitis.
	,,	0·1	**593**	230	,,	15	General tuberculosis.
H 17. "Sp. B." (After increase of virulence).	Rabbit 181, from Calf 539	1·0	**707**	320	Died	16	General tuberculosis.
	,,	1·0	**708**	420	,,	13	,, ,,
	,,	0·1	**709**	530	,,	16	,, ,,
	,,	0·1	**710**	360	,,	25	,, ,,

TABLE SHOWING COMPARATIVE INTRAPERITONEAL INOCULATIONS OF PURE SERUM AND GLYCERIN-SERUM CULTURES OF VARIOUS VIRUSES INTO GUINEA-PIGS.

B.—GLYCERIN-SERUM CULTURES—Viruses virulent for bovines.

GUINEA-PIGS.

Virus.	Immediate Source of Culture.	Dose in Milli-grammes	No. of G.P.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H 38. "J.M." -	G.P. 793, from human cervical glands.	1·0	**584**	440	Died	24	General tuberculosis.
	,,	1·0	**585**	510	,,	22	,, ,,
	,,	0·1	**586**	480	,,	38	,, ,,
	,,	0·1	**587**	560	,,	33	,, ,,
H 32. "Y.W." -	Rabbit 166, from human bronchial gland.	1·0	**594**	250	Died	17	General tuberculosis.
	,,	1·0	**595**	370	,,	15	,, ,,
	,,	0·1	**596**	230	,,	20	,, ,,
	,,	0·1	**597**	240	,,	20	,, ,,
H 17. "Sp.B." - [After increase of virulence.]	Rabbit 181, from Calf 539.	1·0	**703**	450	Died	14	General tuberculosis.
	,,	1·0	**704**	420	,,	16	,, ,,
	,,	0·1	**705**	380	,,	31	General tuberculosis (injection partly subperitoneal).
	,,	0·1	**706**	390	,,	25	General tuberculosis.

TABLE SHOWING COMPARATIVE INTRAPERITONEAL INOCULATIONS OF PURE SERUM AND GLYCERIN-SERUM CULTURES OF VARIOUS VIRUSES INTO GUINEA PIGS.

A. SERUM CULTURES—Viruses slightly virulent for bovines.

GUINEA-PIGS.

Virus.	Immediate Source of Culture.	Dose in Milligrammes	No. of G.P.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H 50. "P.H." -	G.P. 1490 from human lung.	1·	**572**	520	Died	21	General tuberculosis.
	"	1·	**573**	320	"	23	" "
	"	0·1	**574**	420	"	23	" "
	"	0·1	**575**	380	"	27	" "
H 25. "A.T." -	Calf 417 direct	1·	**747**	380	Died	20	General tuberculosis.
	"	1·	**748**	330	"	28	" "
	"	0·1	**757**	320	"	31	" "
	"	0·1	**758**	310	"	29	" "
H 26. "K.M." -	G.P. 1326 from human kidney.	1·	**615**	300	Died	22	General tuberculosis.
	"	1·	**616**	240	"	27	" "
	"	0·1	**617**	320	"	27	" "
	"	0·1	**618**	250	"	24	Tuberculous (injection ? partly intracæcal).

TABLE SHOWING COMPARATIVE INTRAPERITONEAL INOCULATIONS OF PURE SERUM AND GLYCERIN-SERUM CULTURES OF VARIOUS VIRUSES INTO GUINEA PIGS.

B. GLYCERIN-SERUM CULTURES—Viruses slightly virulent for bovines.

GUINEA-PIGS

Virus.	Immediate Source of Culture.	Dose in Milligrammes	No. of G.P.	Weight in grms.	Killed or Died.	Duration of Life in Days.	Result.
H 50. "P.H." -	G.P. 1490, from human lung.	1·	576	420	Died	29	General tuberculosis.
	"	1·	577	480	"	95	General tuberculosis (injection partly subcutaneous).
	"	0·1	578	480	"	28	General tuberculosis.
	"	0·1	579	360	"	28	" "
H 25. "A.T." -	Calf 417, direct	1·	759	330	Died	28	General tuberculosis.
	"	1·	760	480	"	28	" "
	"	0·1	762	360	"	31	" "
H 26. "K.M." -	G.P. 1326, from human kidney.	1·	611	250	Died	25	General tuberculosis. Kidneys affected.
	"	1·	612	250	"	36	General tuberculosis.
	"	0·1	613	260	"	31	General tuberculosis. Kidneys rather extensively affected.
	"	0·1	614	250	"	34	General tuberculosis.

ANALYSIS OF THE PRECEDING TABLES,

Showing the comparative virulence for the Guinea-pig (indicated by the duration of life) of pure serum and glycerin-serum cultures.

A. Viruses Virulent for Bovines.

Dose in milli-grammes.	H 38. "J.M."		H 32. "Y.W."		H 17. "Sp. B."* (3rd Passage.)		Average.	
	S.	G.S.	S.	G.S.	S.	G.S.	S.	G.S.
1·0	14 & 16	24 & 22	16	17 & 15	16 & 13	17 & 15	**15·**	**18·3**
0·1	21 & 21	38 & 33	15	20 & 20	16 & 25	20 & 20	**19·6**	**25·**

B. Viruses Slightly Virulent for Bovines.

Dose in milli-grammes.	H 50. "P.H."		H 25. "A.T."		H 26. "K.M."		Average.	
	S.	G.S.	S.	G.S.	S.	G.S.	S.	G.S.
1·0	21 & 23	29	20 & 28	28 & 28	22 & 27	25 & 36	**23·5**	**29·2**
0·1	23 & 27	28 & 28	31 & 29	31	27	31 & 34	**27·4**	**30·4**

S. = Pure serum cultures. G.S. = Cultures on serum + 5 per cent. of glycerin. * After increase of virulence.

The numbers in the columns headed "S" represent the duration of life in days of guinea-pigs injected with different doses of serum cultures; the opposite numbers in the columns headed "G.S." represent the duration of life of guinea-pigs injected with similar doses of glycerin-serum cultures. The average duration of life of the guinea-pigs inoculated with serum and glycerin serum cultures respectively in each of the two doses used is also shown. All those animals in which the tuberculosis was seriously complicated by other disease, or in which there was evidence that the injection had not been entirely intraperitoneal, are omitted from the analysis.

In these experiments, as in those with the rabbits, the serum cultures were on the whole a little more rapidly fatal than those on glycerin-serum. This difference is equally marked with the viruses of the two groups.

The difference in the duration of the disease caused by serum and glycerin-serum cultures respectively, both in rabbits and guinea-pigs, is not constant, but the longer survival of the animals injected with glycerin-serum cultures occurred too frequently to be altogether the result of accident, and one must therefore conclude that the injection of bacilli grown upon serum cultures causes a slightly more severe disease than that produced by bacilli from glycerin-serum cultures. The difference, however, is too slight to materially affect the results of the experiments made to ascertain the virulence of our viruses.

Does long-continued Cultivation on Glycerin Media attenuate?

Having shown that glycerin-serum cultures are slightly less virulent than serum cultures, the next point to consider is whether the difference in virulence is due to a difference in the chemical products formed in the two media, or whether it is a true attenuation. If the latter, one would expect attenuation to be progressive, that is, to become more marked the longer the culture was kept on glycerin-serum, and one would not expect any return of virulence if the culture were resown on pure serum. If, however, the difference in virulence be due to a difference in the chemical products of bacterial growth on the two media, one would not expect any further change of virulence after the first glycerin-serum culture, as long as the strain remained on that medium, but one would expect a return of virulence if the strain was again grown upon pure serum.

Experiment with the Virus H 10, "B.S."

With the object of investigating these points, a virulent strain of culture was taken, and a series of cultures started on glycerin-serum.

The glycerin-serum cultures were resown every three weeks, and on every alternate occasion of sowing cultures were also sown on pure serum from the last of the glycerin-serum series. Thus one obtained a series of cultures on glycerin-serum, and another on pure serum after growth for three, five, seven and nine generations on glycerin-serum. The latter were injected, when three weeks old, in measured doses, into rabbits, as were also the first and last cultures of the glycerin series. The strain was grown on glycerin-serum for ten generations and during a period of eight months.

The strain used was that belonging to Virus H. 10. "B.S.," and had been obtained from a guinea-pig injected from Calf 113. It had been already grown for thirteen generations on pure serum.

VIRUS H 10. "B.S."

CULTURE DERIVED FROM CALF 113, THROUGH G.P. 757.

[Strain 16½ months old at commencement of experiment]

CULTURES ON PURE SERUM (BOVINE).

Culture 3 weeks old, sown 22-2-05 from pure Serum Culture. Inoculated 15-3-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
298	10·	Died	21	G.T,
297	1·	„	23	G.T.
296	0·1	„	44	G.T.
295	0.01	„	78	G.T.

Sown 17-5-05 on Serum, from the 3rd Culture on Glycerin-Serum.
Inoculated 7-6-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
452	10·	Died	37	G.T. (Injection partly intramuscular).
453	1·	„	13	G.T. Death perhaps hastened by Psorospermosis.
454	1·	„	28	G.T.
455	0·1	„	92	Chronic tuberculosis of Lungs. (Injection intra-cœcal).

Sown 30-6-05 on Serum, from the 5th Culture on Glycerin-Serum.
Inoculated 21-7-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
525	10·	Died	73	G.T. (Injection probably partly intracœcal).
526	1·	„	53	G.T.
527	1·	„	21	G.T.
528	0·1	„	1	Death due to Psorospermosis.

Sown 7-8-05 on Serum, from the 7th Culture on Glycerin-Serum.
Inoculated 28-8-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
555	10·	Died	19	G.T.
556	10·	„	17	G.T.
557	1·	„	24	G.T.
558	1·	„	28	G.T.

Sown 24-10-05 on Serum, from the 9th Culture on Glycerin-Serum.
Inoculated 14-11-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
682	10·	Died	80	G.T. (Injection partly subcutaneous).
683	1·	„	29	G.T. Not very severe.
684	0·1	„	29	G.T.
685	0·01	„	43	G.T. (Injection partly intracœcal).

CULTURES ON 5 PER CENT. GLYCERIN-SERUM (BOVINE).

Culture 3 weeks old, sown 27-4-05, from the 1st culture on Glycerin-Serum. Inoculated 18-5-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
401	10·	Died	17	G.T.
402	1·	„	39	G.T. (Injection partly subperitoneal.)
403	0·1	„	26	G.T.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.

Sown 24-10-05 on Glycerin-Serum, from the 9th Culture on the same medium.
Inoculated 14-11-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
678	10·	Died	16	Early G.T.
679	1·	„	21	G.T.
680	0·1	„	25	G.T.
681	0·01	„	38	G.T.

G.T. = General Tuberculosis.

ANALYSIS OF THE RESULTS OBTAINED WITH THE VIRUS H 10. "B.S."

Dose in Milligrammes.	Serum Culture Control.	2nd Culture on Glycerin-Serum.	Serum Cultures after 3, 5, 7, and 9 generations on Glycerin-Serum.				10th Culture on Glycerin Serum.
			3	5	7	9	
10	21	17	–	–	19 and 17	–	16
1·	23	–	28	53 and 21	24 and 28	29	21
0·1	44	26	–	–	–	29	25
0·01	78	–	–	–	–	–	–

In the analysis of the preceding table the animals are arranged in transverse lines according to doses. The numbers represent the duration of illness (in days). Therefore, if there were any attenuation, the duration of illness should increase as any given line was read from left to right.

The experiment shows that even after having been grown for ten generations on glycerin-serum, and during a period of 8 months, there was no increase in the duration of illness and therefore no attenuation. All those animals in which the tuberculosis was seriously complicated by other disease, or in which there was evidence that the injection had not been entirely intraperitoneal, are omitted. This accounts for the gaps in the analysis.

EXPERIMENT WITH THE VIRUS H 7. "C.M."

A similar experiment was made with the virus H.7, "C.M." It differs from the foregoing in the following particulars. Each culture of the glycerin series was injected into rabbits, and from it a pure serum culture was made, and this also was injected into rabbits. Thus we have a double series of rabbit injections, one with cultures on glycerin-serum, and the other with cultures on pure serum, each after a longer or shorter period of growth upon glycerin-serum.

The strain used for this experiment was that obtained from Calf 5 through Guinea-pig 448, and the series of glycerin-serum cultures was commenced from the 15th culture on pure serum.

VIRUS H. 7 "C.M."

Culture Derived from Calf 5, through G.P. 448.
[Strain 25 months old at commencement of experiment]
Rabbits inoculated intraperitoneally.

CULTURES ON PURE SERUM (BOVINE). Culture 3 weeks old, sown 28-4-05, from pure Serum Culture. Inoculated 19-5-05.

CULTURES ON 5 PER CENT. GLYCERIN-SERUM (BOVINE) Culture 3 weeks old sown, 28-4-05, from pure Serum Culture. Inoculated 19-5-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
408	10·	Died	16	G.T.	**404**	10·	Died	25	G.T.
409	1·	,,	37	G.T.	**405**	1·	,,	37	G.T.
410	0·1	Killed	94	Chronic tuberculosis of lungs and kidneys. (Injection intramuscular and intracœcal.)	**406**	0·1	Killed	94	Chronic tuberculosis rather severe in lungs. (Injection intramuscular and intracœcal.)
411	0·01	Died	39	G.T.	**407**	0·01	Died	94	G.T. of a chronic type.

Sown 19-5-05, from Glycerin-Serum Culture of 28-4-05.

Inoculated 9-6-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
460	10·	Died	12	G.T.	**464**	10·	Died	12	G.T.
461	1·	,,	6	Very early tuberculosis of omentum. Few T.B. in lungs. Death from other causes.	**465**	1·	,,	31	G.T. Lungs more particularly affected.
462	0·1	,,	27	G.T.	**466**	0·1	,,	33	G.T.
463	0·01	,,	47	G.T.	**467**	0·01	,,	47	G.T.

Sown 9-6-05, from Glycerin-Serum Culture of 19-5-05.

Inoculated 1-7-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
505	10·	Died	11	G.T.	**509**	10·	Died	37	G.T.
506	1·	,,	94	G.T. (Injection intramuscular.)	**510**	1·	,,	16	Very slight tuberculosis. Cause of death not determined.
507	0·1	,,	39	G.T.	**511**	0·1	,,	52	G.T.
508	0·01	,,	74	G.T. of a chronic type.	**512**	0·01	,,	98	G.T.

Sown 1-7-05, from Glycerin-Serum Culture of 9-6-05.

Inoculated 22-7-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
533	10·	Died	11	Early G.T.	**529**	10·	Died	5	Early tuberculous peritonitis. Death due to psorospermosis.
534	1·	,,	28	G.T.	**530**	1·	,,	21	G.T.
535	0·1	,,	54	G.T.	**531**	0·1	,,	77	G.T.
536	0·01	,,	1	Psorospermosis.	**532**	0·01	,,	1	Psorospermosis.

Sown 22-7-05, from Glycerin-Serum Culture of 1-7-05.

Inoculated 12-8-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
545	10·	Died	16	G.T.	**541**	10·	Died	25	G.T.
546	1·	,,	87*	G.T.	**542**	1·	,,	30	G.T.
547	0·1	,,	6	Psorospermosis.	**543**	0·1	,,	49	G.T.
548	0·01	,,	94	G.T.	**544**	0·01	,,	142	Chronic Tuberculosis.

Sown 12-8-05, from Glycerin-Serum Culture of 22-7-05.

Inoculated 2-9-05.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.	No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
579	10·	Died	19	G.T.	**583**	10·	Died	17	G.T.
580	1·	,,	33	G.T.	**584**	1·	,,	46	G.T. (injection partly intramuscular.)
581	1·	,,	48	G.T., chronic; injection partly intramuscular.	**585**	1·	,,	19	G.T.
582	0·1	,,	59	G.T. chronic.	**586**	0·1	,,	4	Psorospermosis.

* Needle became detached during injection, and the greater part of the dose was lost.

Note.—"G.T." = General Tuberculosis.

ANALYSIS OF THE RESULTS OBTAINED WITH THE VIRUS H 7. "C.M."

SERUM CULTURES.

Dose.	Serum Culture [Control].	Serum Cultures after 1, 2, 3, 4 and 5 generations on Glycerin Serum.					Average of the last 5 columns.
		1.	2.	3.	4.	5.	
10 mg.	16	12	11	11	16	19	**13·8** days.
1 mg.	37	–	–	28	–	33	**30·5** ,,
0·1 mg.	–	–	39	54	–	59	**50·7** ,,
0·01 mg.	39	47	74	–	–	–	**60·5** ,,

GLYCERIN-SERUM CULTURES.

Dose.	Generation of Culture on Glycerin Serum.						Average.
	1st.	2nd.	3rd.	4th.	5th.	6th.	
10 mg.	25	12	37	–	25	17	**23·2** days.
1 mg.	37	31	–	21	30	19	**27·6** ,,
0·1 mg.	–	33	52	77	49	–	**52·7** ,,
0·01 mg.	94	47	98	–	–	–	**79·7** ,,

The analysis is arranged like the last. All cases complicated by severe psorospermosis or other intercurrent disease are omitted.

It will be observed that the difference in duration of illness between the rabbits which received glycerin-serum and those which received pure serum cultures is far more marked in those which received 10 mg. doses than in those which received smaller quantities. This in itself is in favour of the difference being due to a difference in toxic products. But further it will be seen that no *progressive* diminution of virulence occurred on glycerin-serum and that the serum cultures which were sown from the series of glycerin-serum cultures were as virulent as the original ones which were sown from the strain which had always grown on pure serum.

EXPERIMENT WITH THE VIRUS H 28 'C.L."

If any further evidence is needed to show that glycerin in the culture medium does not attenuate, it is afforded by an experiment with the virus H.28, "C.L." A strain derived from human cervical glands through rabbit 102 was sown on serum and glycerin-serum, and parallel series of cultures on these media were kept growing for over a year. At the end of this period serum cultures were sown from both, and calves and rabbits injected with them. Each strain was injected into two calves in doses of 10 mg. One of these animals unfortunately was found at death to be suffering from obstruction of the ureters. This somewhat interferes with the comparison but it may be stated that each strain proved fatal to one calf, and caused but slight tuberculosis in the other.

In the case of the rabbits the serum culture from the glycerin-serum series actually killed three out of four animals a little quicker than did that sown from the serum series; but this was doubtless a coincidence. One may, however, conclude that the experiment afforded no evidence whatever of the supposed attenuating power of glycerin.

VIRUS H 28. "C.L.'

Strain of culture derived from human cervical glands through Rabbit 102.

CALVES.

SERUM SERIES.

After growing for one year on pure Serum.

A 21-day old serum culture was used for injection.

No. of Calf.	Dose in mg.	Result.		
863	10·	Killed when well,	after 90 days	G.T. slight.
877	10·	Dying*	„ 37 „	G.T. severe.

GLYCERIN-SERUM SERIES.

After growing for one year on Glycerin-Serum.

A 21-day old serum culture, sown from a glycerin-serum culture, was used for injection.

No. of Calf.	Dose in mg.	Result		
875	10·	Killed when well,	after 91 days	Slight tuberculosis
819	10·	Died	„ 52 „	G.T. severe.

* Complicated by obstruction of ureters by calculi.

RABBITS.

SERUM SERIES.

After growing for one year on pure Serum.

A 21-day old serum culture was used for injection.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
559	10·	Died	16	G.T.
560	1·	„	23	G.T.
561	1·	„	23	G.T.
562	0·1	„	31	G.T.

GLYCERIN-SERUM SERIES.

After growing for one year on Glycerin-Serum.

A 21-day old serum culture, sown from a glycerin-serum culture, was used for injection.

No. of Rabbit.	Dose in mg.	Killed or Died.	Duration of Life in Days.	Result.
563	10·	Died	12	G.T. acute.
564	1·	„	24	G.T.
565	1·	„	20	G.T.
566	0·1	„	29	G.T.

SUMMARY.

There can, I think, be no doubt that when grown on pure serum, cultures of viruses belonging to Group I. on the whole prove rather more rapidly fatal to rabbits and guinea-pigs than when they have been grown on serum with glycerin. The difference is most marked with large doses. It is neither progressive nor permanent; strains grown for months or a year on glycerin-serum are no less virulent than the same strain sown for one generation only on that medium. Transference to pure serum restores the original virulence. Therefore, one must conclude that glycerin causes no true attenuation of the bacilli; and that the reason that, dose for dose, serum cultures are a little more rapidly fatal than glycerin-serum cultures is, in all probability, because the latter are deficient in some toxic product which is present in the former.

Note.—G.T. = General Tuberculosis.

REPORT ON THE MINUTE NON-PROGRESSIVE TUBERCLES FOUND IN CALVES INJECTED WITH TUBERCLE BACILLI OF HUMAN ORIGIN WHICH ARE UNABLE TO PROVOKE A PROGRESSIVE INFECTION IN THIS SPECIES OF ANIMAL.

Dr. L. COBBETT.

BLYTHWOOD

REPORT ON THE MINUTE NON-PROGRESSIVE TUBERCLES FOUND IN CALVES INJECTED WITH TUBERCLE BACILLI WHICH ARE UNABLE TO PROVOKE A PROGRESSIVE INFECTION IN THIS SPECIES OF ANIMAL.

In addition to the lesions* constantly found in calves subcutaneously injected with viruses of human group ii, namely, those incapable of causing progressive tuberculosis in these animals there were found on close inspection often one or more, rarely many, minute tubercular foci in the larger viscera or in the lymphatic glands.

In this Report these little tubercles are described, and their incidence among the injected calves is analysed with the object of elucidating their significance. Two questions present themselves :

(1) Are they characteristic of certain viruses, which are thus shown to be of somewhat higher virulence than other viruses which do not produce them ?

(2) Or if not, what determines their presence in some calves, and their absence from others ?

These minute tuberculous foci appeared in the lymphatic glands as opaque, ochre-coloured, calcareous points. They were usually few in number, but in one or two instances they were rather numerous. (*e.g.* in Calf 619, H. 12, H .N.).

Among the great organs they were most commonly found in the lungs, and there appeared as translucent grey points, darker than the surrounding tissue. They were often so small that they were not easily seen, and sometimes they were only found after the lungs had been allowed to dry a little. At most they were as large as a small pin's head. They were either entirely translucent, looking like little specks of glass, or they had a minute central opacity. They were hard, and could easily be picked out on the point of a knife where they appeared as sharply defined spherical bodies resembling little beads of glass, or rather transparent pearls. When crushed on a cover glass they could be distinctly felt to crack.

When these little bodies appeared in the liver or spleen, which they did but rarely, they were similar to those in the lung. Only they then appeared as pale bodies on a darker ground, owing, I suppose, merely to contrast.

They were usually few in number; often two or three only were found in a single organ; but sometimes they were more numerous, and in four instances they occurred in somewhat large numbers.

That these bodies were tuberculous in origin cannot be doubted, because tubercle bacilli were sometimes found in them. That they were not early tubercles capable of progressing there can equally be no doubt, for they were found months after injection in calves which were obviously doing well, and had long ago recovered from any disturbance of health caused by the injection. Histological examination has shown that they were not in an active stage of formation; and there can be no doubt that they were tubercles which had very early commenced to retrogress.

This was not at first clearly recognised, and in one or two calves in which they occurred in relatively large numbers, notably in Calf 365, H. 23, J.P., Calves 165 and 405, H. 18, T.T., and Calf 185, H. 9., C.T., they were mistaken for progressive tubercles in an early stage. Further experience during the last two years has convinced me that I was in error in so thinking, and a recent examination of the preserved specimens has shown how very minute the lesions were in even the more severe cases.

These four calves are therefore now regarded by me as showing in their organs the minute lesions of retrogressive type, so frequently seen in calves injected with viruses which are not capable of causing in them a progressive tuberculosis, and only exceptional in possessing them in unusual numbers.

The origin of these little tubercles seems to be attributable to bacilli which passively escape from the seat of subcutaneous injection. For the evidence of this the report on the distribution of non-virulent tubercle bacilli in the body of the calf after subcutaneous injection may be consulted [*See* p. 219]. In that Report it is shown that after subcutaneous injection, even of viruses incapable of causing a progressive infection, tubercle bacilli get distributed all over the body, and that, as a rule, they disappear without leaving any sign of their former presence. Occasionally, however, the minute translucent foci are found three months after injection, and may even then contain a few tubercle bacilli.

These little tubercles are evidence that the disease has made some amount of progress at a number of foci distant from the seat of injection, however completely that progress may have ceased before the animal came to be examined. The question then may be put thus : Is this short-lived development of tubercles in glands and organs rendered possible by any special quality of virulence on the part of the bacilli, or by some peculiar susceptibility of the individual animals in which they appear ? If the former, then one would expect these little tubercles to occur after the injection of certain viruses rather than of others. If the latter then one would expect the calves in which they occur to be distributed equally among all the feebly virulent viruses ; or rather as equally as the laws of probability will allow.

To answer this question then one needs to know whether any of the feebly virulent viruses are conspicuous among others for their power to cause these little tubercles, or whether all feebly-virulent viruses alike are liable to produce them. Accordingly a table has been drawn up including all the calves which have up to the present time shown these lesions, and a list giving for each virus the proportion of animals so affected to the total number injected subcutaneously with it.

In the table the tubercles in the organs are distinguished from those in the glands, and among the latter, the thoracic, mesenteric, and other lymph glands distant from the seat of injection are recorded separately. Moreover, the numbers of tubercles in each of these groups is roughly indicated : thus two or three or very few such foci are indicated by a single x, moderate and large numbers by x x and x x x respectively.

The table shows that these little tubercles have occurred in one or more of these situations and in larger or smaller numbers in nearly half the calves injected subcutaneously with viruses of group ii, and that these calves are widely distributed. All the viruses which have never produced progressive tuberculosis in bovine animals have caused these lesions, with the exception of seven (out of thirty-one). With each of these no more than two calves were used ; when larger numbers were injected with one virus some have always been affected, and the ratio of these to those which escaped is remarkably constant.

If we separate the viruses according to their seat of origin in the human being we find that no category is particularly distinguished for producing them, and that instances in which they were especially numerous occur among the lung, mesenteric gland, and joint viruses.

On the other hand it is only right to point out that the incidence of these little tubercles is rather greater among the calves injected with cervical and mesenteric gland

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(*) These consisted of a caseous and softened local lesion enclosed in a fibrous capsule, and of calcareous nodules in the nearest lymphatic glands ; often of nothing more. These caseous abscesses often ulcerated through the skin and discharged their content. The latter must have often been swallowed by the calves, and yet lesions attributable to this cause were seldom seen. The lesions in Calves 473 and 481, H. 30, E.M., may have had this origin.

viruses than among those injected with lung or joint viruses; and these latter, it will be remembered, have, unlike the former, never yet yielded one virulent for cattle. But the difference is but slight (41 and 42·4 as compared with 50 per cent.), and it may be doubted whether this is more than accidental. On the whole, then, the conclusion seems justified that these little tubercles are not evidence of a superior grade of virulence in the virus that produced them.

The next question is: Why do these little tubercles occur in some animals and not in others injected with the same virus? The explanations which suggest themselves, namely, variations of dose, and individual susceptibility, and some accidental peculiarity of the mode of injection, will now be considered.

Dose we may dismiss at once, because the lesions have been caused by all kinds of doses, having followed such small ones as 13,000 and 10,000 bacilli in the case of H. 16 "J.H." and H. 12 "H.N." and having been absent after such colossal doses as 5,000 million (H. 16 "J.H"), 3,000 million (H 12 "H.N."), as well as 10,000 and 5,000 million (H. 26 "K.M."), and 5,000 million (H. 8 "S.C.").

As to individual susceptibility, there can, I think, be no doubt that great differences occur among calves. Of this there is a large amount of evidence, and I will only mention now the results of the 10 m.g. culture injections, which, in the case of two calves injected with the same virus, have often proved rapidly fatal to one, and left the other to live out its three months, without serious illness, and with a very moderate amount of disease at the end of this period. These injections were made with virulent viruses, but it seems reasonable to suppose that the same differences of susceptibility would come into play in the case of the feebly-virulent viruses also. (*)

But while differences of individual susceptibility appear as the most probable explanation of these little tubercles, I think I ought to point out that there are differences also in the circumstances of subcutaneous injections which may be not without influence. The possibility of some of the material entering a vein at the moment of injection cannot be seriously entertained. I desire rather to refer to differences which depend on the exact depth of the injection. Thus on occasions the injection has been made partly into the muscle, and even when this is not the case, differences in the plane of subcutaneous connective tissue into which the fluid enters may cause great differences in the amount of tension to which it is subjected. Thus, for example, at times one experiences a good deal of resistance to the entry of fluid, and in such cases the little swelling which results is prominent, circumscribed, and very tense; at other times there is little or no resistance and the swelling is flat and soft. Again the fluid may run up a considerable distance from the point of the needle, forming an elongated cord-like swelling. This happens, I think, when the needle enters the sheath of the subjacent muscle. In these varying circumstances it does not seem unreasonable to think that the difference of tension, at least, may make some difference to the passive absorption and consequent dissemination of tubercle bacilli.

However this may be, it seems obvious that each of these little tubercles must develop at a spot where a considerable number of tubercle bacilli have become arrested, for the report, on the distribution of bacilli already referred to, made it clear that the little tubercles by no means always develop wherever the bacilli have wandered to. Considerable numbers of bacilli at a single spot seem only explicable on the hypothesis that a phagocyte with a large cargo of them had run aground there. Probably wherever isolated bacilli, or small groups are arrested they are destroyed. But in the case of these larger aggregations some development of new tissue (tubercle formation), takes place before the invaders are destroyed. Thus the existence of these tubercles would appear to depend upon the presence of overloaded phagocytes, together with the inability of the animal to promptly destroy the bacilli. Whatever be the significance of the former, the latter is the result of some defect of the protective functions of the body. But it is hard to say which is the dominant factor.

Louis Cobbett.

(*) A fuller statement of the evidence of differences of individual susceptibility will be found in the Report on the influence of (i) the variation in the capacity for resistance to tuberculosis of different individual animals, and (ii) the number of tubercle bacilli injected, on the severity of an experimental infection [*See* p. 247].

LIST OF CALVES INJECTED SUBCUTANEOUSLY WITH VIRUSES OF GROUP II., IN WHICH LITTLE NON-PROGRESSIVE TUBERCLES WERE FOUND.

X = One or more minute foci. XX = Fairly numerous minute foci. XXX = Very numerous minute foci.

Virus. [The probable primary seat of disease in man is indicated in brackets].	No. of Calf.	Tissue Emulsion or Culture.	Dose.	Non-progressive minute Tubercles in			
				Organs.	Thoracic Glands.	Mesenteric Glands.	Other distant Glands.
H. 17. "Sp.B."† [Lung] -	265	T.	50,000	X	X		
	277	T.	76,000	X	X		
	391	T.	41,600,000	X	X		
	601	C.	50 mg.			X	
H. 22. "F.W." [Lung] -	359	T.	3,204,000		X		
	439	T.	100,000,000		X		
	755	C.	50mg.	X			
	759	C.	50mg.	X			
H. 23. "J.P." [Lung] - -	365	T.	103,093,000	XXX	X	X	X
	421	T.	8,166,000	X	X	X	
	625	C.	50mg.	X	X	X	X
H. 25. "A.T." [Lung] - -	449	T.	87,273,000		X		X
H. 50. "P.H." [Lung] - -	843	C.	50mg.	X		X	
H. 51. "H.M." [Lung] - -	833	C.	50mg.	X			
H. 45. "F.M." [Bronchial gland]	763	C.	50mg.	X		X	
H. 35. "C.B." [Bronchial gland]	605	C.	50mg.	X	X	X	
H. 27. "B.D." [Cervical gland]	675	C.	50mg.	X			
	677	C.	"	X			
H. 37. "O.J." [Cervical glands]	627	C.	50mg.	X			
	629	C.	"			X	
H. 44. "D.C." [Cervical glands]	705	C.	50mg.	X		XX	
	731	C.	"		X	XX	XX
H. 33. "R.T." [Cervical glands]	595	C.	50mg.	X			

† Before increase of virulence.

LIST OF CALVES INJECTED SUBCUTANEOUSLY WITH VIRUSES OF GROUP II. IN WHICH LITTLE NON-PROGRESSIVE TUBERCLES WERE FOUND—*continued.*

Virus. [The probable primary seat of disease in man is indicated in brackets.]	No. of Calf.	Tissue Emulsion or Culture	Dose.	Non-progressive minute Tubercles in			
				Organs.	Thoracic Glands.	Mesenteric Glands.	Other distant Glands.
H. 8. "S.C.' [Mesenteric glands].	275	T.	12,550,000		X		
	323	T.	,,		X		
	423	T.	6,146,000		X		
	657	C.	50mg.	X			
	673	C.	50mg.	X			
H. 12. "H.N." [Mesenteric glands].	123	T.	13,000	X	X		X
	135	T.	,,	X	X	X	
	287	T.	103,968,000	X	X	X	X
	329	T.	2,766,000		X		
	351	T.	3,140,266,000		X	X	
	619	C.	50mg.		X	XX	XX
H. 18. "T.T." [Mesenteric glands].	165	T	4,479,000	XXX	X	X	
	405	T	269,917,000	XXX	T.B.	T.B	
	429	T	2,814,000,000	X		X	
	645	C	50 mg.	X			
H. 30. "E.M." [Mesenteric gland].	473	T	4,756,800,000			XXX	
	481	T	,,	X	X	XXX	X
	593	C	50 mg.	X	X		
H. 36. "M.D." [Mesenteric glands].	687	C	50 mg.	XXX		X	
H. 46. "H.W." [Knee-joint]	777	C	50 mg.			X	
H. 47. "S.B." [Hip-joint] -	803	C	50 mg.	X		X	
H. 9. "C.T." [Wrist joint] -	183	C	¾ c.c. of bacilli.		X		
	185	C	,,	XXX	X		X
	201	T	3,883,000		X		
	331	T	133,824,000	X		X	X
	733	C	50 mg.			X	
	737	C	50 mg.	X	X	X	
H. 11. "E.D." [Elbow joint]	307	T	479,733,000	X	X		
	723	C	50 mg.				

List of Calves Injected Subcutaneously with Viruses of Group II. in which Little Non-progressive Tubercles were found—*continued*.

Virus. [The probable primary seat of disease in man is indicated in brackets].	No. of Calf.	Tissue Emulsions or Culture.	Dose.	Non-progressive minute Tubercles in			
				Organs.	Thoracic Glands.	Mesenteric Glands.	Other distinct Glands.
H. 15. " I.W." [Ankle-joint]	193	T.	3,761,000	X	X		
	195	T.	,,		X		
	367	T.	68,166,000	XX	X	X	
	413	T.	136,960,000		X	XX	
H. 16. " J.H."* [Knee-joint]	157	T.	10,000		X		
	527	C.	50mg.			X	
H. 26. " K.M." [Kidney] -	437	T.	4,936,000	X	X		
	865	C.	50mg.	X		X	
	871	C.	,,	X		X	X

* Before increase of virulence.

TABLE SHOWING THE DISTRIBUTION OF THE CALVES WITH DISSEMINATED NON-PROGRESSIVE TUBERCLES AMONG THE DIFFERENT VIRUSES INCAPABLE OF CAUSING PROGRESSIVE TUBERCULOSIS IN BOVINE ANIMALS (Group II).

Virus.		Ratio of Number of Calves with disseminated tubercles to total number injected with each virus.	
Name.	Primary in.		
H. 17. " Sp.B." *	Lung	4 out of 10	14 out of 33, or 42·4 per cent.
H. 22. "F.W."	„	4 „ 7	
H. 23. "J.P."	„	3 „ 7	
H. 25. "A.T."	„	1 „ 4	
H. 48. "W.P."	„	0 „ 2	
H. 51. "H.M."	„	1 „ 1	
H. 50. "P.H."	„	1 „ 2	
H. 45. "F.M."	Bronchial gland	1 „ 2	2 out of 4, or 50 per cent.
H. 35. "C.B."	„	1 „ 2	
H. 27. "B.D."	Cervical gland	2 „ 4	7 out of 14, or 50 per cent.
H. 37. "O.J."	„	2 „ 2	
H. 34. "C.U."	„	0 „ 2	
H. 39. "M.B."	„	0 „ 2	
H. 44. "D.C."	„	2 „ 2	
H. 33. "R.T."	„	1 „ 2	
H. 8. "S.C."	Mesenteric glands	5 „ 10	19 out of 38, or 50 per cent.
H. 12. "H.N."	„	6 „ 11	
H. 18. "T.T."	„	4 „ 9	
H. 30. "E.M."	„	3 „ 4	
H. 36. "M.D."	„	1 „ 4	
H. 42. "M.R."	Joint	0 „ 2	16 out of 39, or 41 per cent.
H. 43. "F.F."	„	0 „ 2	
H. 46. "H.W."	,	1 „ 1	
H. 47. ‘ S.B."	„	1 „ 2	
H. 41. "A.S."	„	0 „ 2	
H. 9. "C.T."	„	6 „ 7	
H. 11. E.D."	„	2 „ 6	
H. 15. "I.W."	„	4 „ 9	
H. 16. "J.H."*	„	2 „ 8	
H. 40. "J.G."	Testicle	0 „ 2	
H. 26. "K.M."	Kidney	3 „ 6	

Total Incidence Ratio—all Viruses : 60 out of 136, or 44·1 per cent.

* Before increase in virulence.

REPORT ON THE DISTRIBUTION OF NON-VIRULENT TUBERCLE BACILLI OF HUMAN ORIGIN IN THE BODY OF THE CALF AFTER SUBCUTANEOUS INJECTION.

Dr. L. COBBETT.

REPORT ON THE DISTRIBUTION OF NON-VIRULENT TUBERCLE BACILLI IN THE BODY OF THE CALF AFTER SUBCUTANEOUS INJECTION.

The dissemination of a disease such as tuberculosis throughout the body from a focus of infection has generally been pictured as an active invasion by the microorganisms, which in virtue of their virulence break down step by step the successive barriers which are raised against them. This report produces evidence of another kind of dissemination, and describes a passive escape of non-virulent tubercle bacilli from the seat of a subcutaneous injection in the calf, occurring very shortly after that injection, and a wide distribution of the bacilli throughout the body, followed either by their complete disappearance, or by minute lesions only which show no tendency to progress.

These minute lesions have been described in the preceding report, and I need not say more here than that they were either fibrous and very translucent, or calcareous, and so small as to be easily overlooked. They were therefore undoubtedly retrogressive.

Now, these little tubercles, occurring from time to time in the organs of calves which were obviously doing well, were a source of perplexity to me, for I could not understand how the bacilli could have got to these situations unless they possessed the virulence which should have enabled them to cause progressive lesions. Their presence conflicted with the belief that the severity of an infection caused by tubercle bacilli could be measured by the wideness of distribution of the lesions which they produced. For in these cases the distribution had obviously been wide and yet the disease had not progressed, the animals had recovered from any transient disturbance of health from which they once suffered, and had remained well, and grown normally.

Various hypotheses suggested themselves—the bacilli were perhaps in these cases of somewhat higher virulence than the average of the non-virulent class; or the animal exceptionally susceptible. But neither hypothesis reconciled the wideness of distribution with the arrest of progress. Either non-virulent bacilli had succeeded in distributing themselves over the body, or virulent bacilli had become arrested in their activity after they had succeeded in spreading themselves. If some sudden accession of resistent power were known to occur in tubercle as in pneumonia, this latter hypothesis would not have appeared improbable, but in tuberculosis such augmentation of resistance is unknown, and moreover other evidence showed that the viruses in question were of the ordinary non-virulent kind. Gradually it came to appear more and more probable that the dissemination of tubercle bacilli over the body in our experimental animals is not necessarily the active progressive invasion that it has been pictured, but might take place also by a passive absorption, which probably occurs before a barrier of granulation tissue has been built around the bacilli at the seat of injection, and that in the case of the bacilli injected being of feeble virulence for the animal, they either entirely disappear, or where most numerous cause evidence of their presence in a few little glassy or calcareous foci.*

It remained to put this theory to the test of experiment. Now it is obvious, if tubercle bacilli escape in numbers from the seat of injection, and after dissemination about the body disappear without causing any serious lesions, that one must expect the tubercle bacilli to be more numerous in the organs and tissues at some period not long after the injection than at the time when these animals are usually killed, namely three months later. This was actually found to be so in the case of Calf 769, which died of diarrhœa seventeen days after the subcutaneous injection of 50 mg. of a culture of the group II. virus H. 45, "F.M." At the post-mortem examination of this animal an inflammatory patch was found at the seat of injection, and the prescapular and prepectoral glands on the same side were swollen but showed no distinctive signs of tubercle. A scraping from each however, revealed numerous tubercle bacilli, while some were found also in the posterior-thoracic gland, which was normal in appearance. Four films from the lungs were searched but no tubercle bacilli seen. The full notes of the post-mortem examination of this and other calves mentioned here will be found on pages 229–233. At this stage I consulted with Dr. Eastwood, and asked him to cooperate with me in the investigation. To this he cordially agreed, and it was arranged that he should examine histologically the various tissues and organs of the calves while I injected tissue emulsions into guinea pigs. It was obvious that my part of the work could only be expected to show the presence or absence of bacilli in the tissues investigated, and would reveal but little as to their numbers; and it was hoped that the histological investigation would throw light on the method of dissemination which we expected to demonstrate, and by following the numbers of bacilli found in various organs of calves injected simultaneously and killed in succession, give valuable information as to the rise and fall of this dissemination of bacilli, and the relation between their escape from the seat of injection and the development of the local tumour.

Experiments with Virus H. 46.

At this time there happened to be two calves which had recently been injected subcutaneously with 50 mg. of the non-virulent virus H. 46, "H.W." (from a case of joint tuberculosis). In order to get to work as soon as possible it was decided to kill one of these animals about three weeks after injection, when the febrile reaction and the enlargement of the prescapular gland might be expected to be near their maximum, leaving the other as a control, to be killed and examined at the expiry of the usual three months interval.

The first of these calves, No. 767, was accordingly killed on May 23, 1905, twenty-five days after injection, while the second, No. 777, was killed on July 27, and their various organs and tissues were examined in the way agreed upon. Dr. Eastwood's report on this and the other calves which bear on the question under consideration will be found in Volume IV., pp. 13–19. It remains for me to describe the gross lesions of the calves, and the result of injecting the tissue emulsions into guinea-pigs, and as the former are fully given at the end of this report, and the latter clearly shown in the table which follows, it will be unnecessary to give any more than a brief description here.

These two calves, Nos. 767 and 777, had been subcutaneously injected with 50 mg. of three weeks' old serum culture on the right side of the neck on April 28th, 1905.

No. 767 was killed on May 23rd, twenty-five days after injection, when the febrile reaction was just over and the enlargement of the prescapular gland probably at its height. At the postmortem examination there was found a large flat tumour at the seat of injection, caseonecrotic, and containing a large irregular cavity. It had not however begun to soften in its periphery. It was closely attached to the subjacent muscle and slightly infiltrated the skin. The right prescapular gland was enlarged and consisted partly of a grey translucent tissue and partly of a caseating network. The right prepectoral gland was slightly affected. On the surface of the lungs rather numerous minute translucent grey points could be seen. Some of these had a reddish tinge, and it was difficult to distinguish them from the minute hæmorrhages which are of frequent occurrence, even in normal animals. Some were undoubtedly tubercles of the kind frequently found in these cases, for they could be picked out on the point of a knife. Scarcely any trace of opacity could be seen in the centres of any of them. In the liver and kidneys one or two pale specks on the surface were the nearest approach to tubercles which could be seen. All other parts appeared perfectly normal.

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* At one time one questioned whether in these cases some of the injected material might not have got into a torn vein at the moment of injection, but the repeated occurrence of such cases made this suggestion unthinkable.

Emulsions were made from liver, spleen, lung, and rib marrow, all but the last being rich in tissue, and 1·0 cc. of each was injected into guinea-pigs. Table showing the results of these and similar inoculations will be found at the end of this report.

All the guinea-pigs were found to be tuberculous when killed seven weeks later. One alone, injected with marrow, had tuberculosis of the omental gland only, all the rest had general tuberculosis. There was obviously then a very wide distribution of tubercle bacilli in this calf, involving the bone marrow as well as the principal organs, far wider than the visible lesions would have led one to suppose.

The control Calf, No. 777, was killed ninety days after injection. The lesion at the seat of injection consisted of a fibrous walled cavity with fluid contents, and the prescapular gland on the same side contained two large fibro-calcareous nodules. In several of the mesenteric glands were some small calcareous foci. There was no visible trace of tubercle elsewhere, or vestige of the previous presence of bacilli, unless indeed some little areas of collapse in the lung were of this kind.

Guinea-pigs were injected with emulsions of the same parts as before, care being taken to inject even larger doses. The guinea pigs which received emulsions of spleen, liver, and rib marrow intraperitoneally were perfectly healthy when killed forty-two days later, while those injected with the lung emulsion were found to be tuberculous.

Experiment with Virus H. 26.

The next experiment was made with the virus H 26, "K.M." (obtained from a human kidney), which had proved itself non-virulent for calves and rabbits, and of which the enormous dose of 10,000 million bacilli had already been injected into a calf with only minimal results.

On July 25, 50 mg. of three weeks' old serum culture was injected into each of five calves subcutaneously on the right side of the neck.

No. 873 was killed *three* days later. There was a small soft swelling at the seat of injection, the subcutaneous tissue being a little thicker and firmer than normal, and the surrounding tissue a little œdematous. The prescapular gland on the same side was somewhat swollen and œdematous; everything else appeared perfectly normal.

Some 3 or 4 c.c. of blood from the left iliac vein were injected into a guinea pig, without producing tuberculosis. Tissue emulsions of spleen, liver, and lung, as well as of bone marrow and popliteal gland (taken as a representative of the peripheral lymphatic glands) caused tuberculosis in the guinea pig. Only in the case of the animal injected with the popliteal gland was the tuberculosis slight, in all others it was general. The doses were large, and the emulsions with the exception of that of the bone marrow were rich in tissue.

Calf 881 was killed *eight* days after the injection. There was a small swelling at the seat of injection chiefly caused by a thickening of the subcutaneous tissue, which was firm and grey and infiltrated with shot-sized spots of softened matter resembling muco-pus. The right prescapular gland was somewhat swollen, but showed no sign of tubercle. All other parts were normal.

The tissue emulsions of this calf which were injected into guinea pigs were more numerous than before, and a larger dose of blood was given. The only guinea pig which escaped tuberculosis was one of two which were injected with an emulsion of rib marrow, the other was tuberculous. That which received 11 c.c. of blood from the heart was found when killed to have six tubercles in its spleen. Those injected with emulsion of spleen, liver, lung, kidney, thoracic gland, and popliteal gland, all were found to have general tuberculosis. The emulsions, with the exception of that of the marrow, were rich in tissue, and large doses were given.

The third calf, No. 869, was killed *fourteen* days after injection. It had remained quite well, but its temperature had risen a little, (to 40·2°C.) on the 13th day. It had gained 9lbs. in weight.

At the seat of injection was a tumour which measured 4 × 3 × 1¼in. In its centre was an irregular cavity containing muco-purulent material around which was dense œdematous tissue; the subjacent muscle was not infiltrated, but the skin over the tumour was distinctly altered in appearance. The prescapular gland was considerably enlarged. It was firm, elastic and œdematous, but there was no appearance characteristic of tubercle. The prepectoral was as large as a large pea, very similar in appearance to the prescapular but yellower. All other organs and glands were normal in appearance.

Injections of tissue emulsions were made into guinea pigs as before. Between 2 and 3 c.c. of blood only was injected and this caused no infection. The rib marrow again infected one guinea pig only out of two; the spleen, liver, lung, kidney, long thoracic and popliteal glands caused general tuberculosis; the doses were large and the emulsions except that of the rib marrow rich in tissue as before.

The two remaining calves were killed three months after injection. No. 871 had developed a very large swelling and gland, and had been obviously ill a fortnight after the injection, with rapid respiration, staring coat, and high temperature lasting two or three weeks. Later on it became thin, but afterwards picked up, and gained 27 lbs. The average rate of gain during the whole period had been 2lbs. per week. It had been quite well for a month or more before it was killed.

At the seat of injection there was the thin-walled fluid tumour characteristic of a non-virulent injection. There was no infiltration of skin or muscle. The prescapular gland was a little enlarged and almost entirely occupied by a calcareous mass. The prepectoral was also enlarged and very similar in appearance. There were a good many little glassy tuberculous foci in the lungs, few of which had any central opacity, and two rather larger shot-sized tubercles. There were one or two very minute pale specks in each kidney. The suprarenals each contained one or two small tubercles. Minute calcareous foci were found in small numbers in the bronchial and other thoracic glands; the mesenteric, the left supra-pharyngeal and submaxillary, both precrural, popliteal, gluteal, and ischiatic glands. So wide a distribution of non-progressive tubercles is unusual, but other examples have occurred, for example, in 619, H. 12, "H.N."

Tissue emulsions were made and injected into guinea pigs as before. Those injected with blood (13cc). died in a few hours, and one of those injected with marrow died in two days. The rest were killed after fifty-three days (*see* table.) The second guinea-pig injected with marrow was healthy, as were those injected with kidney. Those injected with lungs, liver and spleen had tuberculosis more or less generalised, but in no case severe. These guinea pigs were less affected than those which received emulsions from the more recently affected calves. One may therefore conclude that the bacilli had disappeared from the marrow and kidney, but were still present, though in reduced numbers in the lungs, liver and spleen. The presence of living non-virulent bacilli in the organs other than the lungs after three months must, I think, be regarded as exceptional, judging from Calf 777, "H.W.", and 865 "K.M." which follows, and remembering that the animal in question presented an unusual number of minute lesions visible to the naked eye.

Calf 865 was killed about the same time. It had been well almost ever since the injection, except for a transient disturbance of health about the third week, and a period of slight fever which lasted about a fortnight. Its average rate of gain during the whole period was 4 lbs. per week. The tumour at the seat of inoculation had softened, ulcerated, and discharged its contents.

At the seat of injection was found a little depressed opening in the skin, leading to a patch of dense brownish-pink tissue packed with little calcareous grains.

The prescapular gland on the same side was a little enlarged and contained some large calcareous nodules, one of which was commencing to soften. The right prepectoral was normal. In the mesenteric glands a little yellow calcareous focus was found here and there: everything else was normal, unless indeed some little grey specks in the lungs were the legacy of the tubercle bacilli which had been there. They were more minute by far than the glassy tuberculous foci so often described, and they would not have been thought worthy of mention in any other case.

Tissue emulsions were made as before and injected into guinea pigs, care being taken that the doses should be larger than those used in the three first calves of this experiment. Those injected with blood (12cc.) died in a few hours. One guinea-pig injected with lung died after nine days and showed no signs of tuberculosis. One of those injected with popliteal gland died after fifty days, and was found to have general tuberculosis. The remaining guinea-pigs were killed on the 51st day. The one injected with lung was found to have slight tuberculosis, thus supporting the suspicion that the very minute grey specks seen in that organ at the post-mortem examination of the calf were tuberculous in origin. Those injected with spleen, liver, kidney, and rib marrow were found to be perfectly healthy. On the other hand all those injected with glands, namely the popliteal, long thoracic, bronchial and hepatic were tuberculous.

The presence of tubercle bacilli in some of the apparently healthy organs and glands of these two calves, and of Calf 777, which, it will be remembered, had been injected with a virus incapable of producing a progressive, and indeed anything more than a minimal tuberculosis in bovine animals, and which had recovered from any temporary illness from which they may have suffered when they were killed 3 months after injection, was surprising. It became clear that non-virulent tubercle bacilli which get passively disseminated after a subcutaneous injection do not entirely disappear within 3 months.

On the other hand a comparison of the distribution of bacilli in these animals with that in those killed a few days or weeks after injection will leave no doubt that bacilli so disseminated become less numerous as time goes on, and although they have not everywhere disappeared at the end of 3 months, they at least have done so from many parts of the body.

It has been said that one of the calves killed after three months suffered from an illness of unusual severity as compared with other animals injected with similar doses of viruses of Group II. It is possible, therefore, that the distribution of bacilli and their persistence in this animal was greater than usual; and it may be interesting to collect together in a table all the other guinea-pig injections made from time to time with the organs of bovine animals which had been injected with viruses of Group II. (*See* page 228.)

These injections were made for various purposes; some with the object of finding out whether a suspicious focus was tuberculous or no; some with the direct intention of ascertaining whether or no bacilli were to be found in apparently healthy organs; and others again—not always successfully—to demonstrate the non-tuberculous origin of some intercurrent disease, such as pneumonia, or carnification of the lung.

It will be seen that the presence of tubercle bacilli in these organs was by no means uncommon. Probably, however, the frequency is somewhat exaggerated by these experiments which were made, not in every case, but exceptionally for one of the reasons given above, consequently the animals from which the emulsions were taken are not quite fair samples of those injected with viruses not virulent for bovines, but were, some of them at least, selected on account of the presence of more or less suspicious foci, or for some other reason.

However, when one has made all due allowance for this, the findings of tubercle bacilli are still numerous, and one cannot doubt *that tubercle bacilli are frequently present in the organs and glands of bovine animals several months after they have been injected with bacilli which belong to the human Group II. Often they are contained in minute retrogressive tuberculous foci; but not unfrequently in organs which appear perfectly healthy to the naked eye.*

The negative result obtained in the case of the few experiments made with the organs of animals infected by feeding, seems to suggest that passive dissemination of bacilli does not take place in the absence of solution of continuity of tissue.

SUMMARY AND CONCLUSIONS.

The little glassy or calcareous tubercles often seen in the lungs, and more rarely in other organs of calves injected with tubercle bacilli which failed to produce in them a progressive infection, gradually led to the conviction that there must be *a passive* escape of tubercle bacilli into the circulation; for if they could only have made their way to these distant parts by *active invasion*, that is by overcoming the resistance offered to them by the animal organism, it seemed impossible to understand why they should then have failed to develop there. These experiments have revealed the presence of tubercle bacilli practically all over the body within three days of a subcutaneous injection of 50 mg. of a non-virulent culture, thus demonstrating the fact of passive distribution which had been inferred from the presence of these little glassy tubercles, and at the same time explaining their existence and showing that they afforded no evidence of virulence, and were in no sense due to the *active* invasion of the body by the bacilli which caused them.

It is not my intention to discuss the mode of dissemination, except to say that ultimately it must be by the blood. The very wideness of distribution points to this, and in one calf indeed, the blood from the heart itself infected a guinea-pig. Whether the escape of the bacilli from the seat of injection is by the lymphatic channels, and whether they are simply washed away in the stream of fluid, or carried by phagocytes are questions which fall rather into Dr. Eastwood's province than mine. But I should like to refer to one point which may be thought to have some bearing on the question, namely the difference between the local lesion produced by a virulent and that by a non-virulent virus. One of the characteristic features of the tumour caused at the seat of a subcutaneous injection in the calf by a virulent virus is that it widely infiltrates surrounding tissues, and innumerable caseous foci are found in the neighbouring skin, while the muscle often for a considerable distance contains caseous streaks and patches. On more than one occasion the lymphatics connecting the seat of injection in the neck with the nearest prescapular gland have been found thickened to the size of a small quill by a caseous deposit. On the other hand, if the virus is of the non-virulent kind the tumour after three months is sharply circumscribed and encased in a fibrous capsule often of considerable thickness, and with this capsule it can be easily dissected away from surrounding structures. One cannot but be reminded of the similar difference between a malignant and a benign tumour.

There seems no reason to doubt that the infiltration in the former case takes place by that process of active invasion which was alluded to at the opening of this report, and the fact that in the case of injection of *non-virulent* bacilli the tumour is sharply circumscribed seems to show that this *active* dissemination does not take place unless the virus is virulent, and that the little visceral lesions, which may follow the injection of a non-virulent virus, must be due to an absorption which is purely passive.

The passive distribution of tubercle bacilli from the seat of injection, appears to me to afford a reasonable explanation of the influence of dose in determining the severity of an infection caused by micro-organisms subcutaneously injected. For it is obvious that the numbers of bacilli *passively* distributed in the manner here described will depend largely upon the quantity injected. Whereas on the other hand, if they could only get out by a process of *active invasion* the numbers which thus gained access to distant parts would depend mainly on their activity or virulence, and but little on the original numbers injected; and yet we have repeatedly seen the injection of small numbers, even of very virulent bacilli, fail to cause a general infection.

It is not suggested that a dissemination of micro-organisms, comparable to what takes place when large doses of culture are *injected*, necessarily plays an important part in tuberculous, or other infections under natural conditions, when the number of invading bacilli must necessarily be small. It is not my intention to discuss here the question, whether it ever plays any, and if so what part under these conditions. The interest of the observations recorded in this report lie, at the moment, in the light which they throw on the consequences of artificial infections such as are constantly being produced here. But the explanation which they offer of the occurrence of apparently isolated tuberculous lesions in distant parts, *e.g.* in the hip, will not be overlooked.

LOUIS COBBETT.

GUINEA PIGS INOCULATED WITH VARIOUS TISSUES OF

Calf 767 (H. 46, "H.W.").

[Killed 25 days after injection.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose.	Duration of Life, in Days.	Killed or Died.	Result.*
1544	E. of Liver - - -	1·0 cc.	49	Killed.	G.T.
1545		"	"	"	"
1546	E. of Spleen - -	"	"	"	"
1547		"	"	"	"
1548	E. of Lung - - -	"	"	"	"
1549		"	"	"	"
1550	E. of Rib Marrow - -	"	"	"	T. of Omental Gland only.
1551		"	"	"	G.T.

These emulsions, with the exception of that from the rib marrow, were very rich in tissue.

GUINEA PIGS INOCULATED WITH VARIOUS TISSUES OF

Calf 777 (H. 46, "H.W.").

[Killed 90 days after injection.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose.	Duration of Life in Days.	Killed or Died.	Result.
1619	E. of Lung- - - -	2·0 cc.	42	K.	G.T.
1620		"	"	"	"
1621	E. of Spleen - -	"	"	"	Healthy.
1622		"	"	"	"
1620	E. of Liver - - -	"	"	"	"
1624		"	"	"	"
1625	E. of Rib Marrow - -	1·0 cc.	"	"	"

These emulsions, with the exception of that from the rib marrow, were very rich in tissue.

* T. = Tuberculous or Tuberculosis.
S.T. = Slight Tuberculosis.
G.T. = General Tuberculosis.
No T. = No Tuberculosis.

Guinea-Pigs inoculated with various Tissues of

Calf 873 (H. 26, "K.M.").

[Killed 3 days after injection.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose.	Duration of Life, in Days.	Killed or Died.	Result.
1626	E. of Popliteal Gland - -	7·0 cc.	45	Killed	Slight G.T.
1627	E. of Rib Marrow - -	3·0 to 4·0 cc.	"	"	G.T.
1628	E. of Spleen - - -	10·0 cc.	"	"	"
1629		"	"	"	"
1630	Blood from left Iliac Vein -	3·0 to 4·0 cc.	"	"	Healthy.
1631	E. of Liver - - - -	6·0 cc.	"	"	G.T.
1632		"	"	"	"
1633	E. of Lung - - - -	15·0 cc.	"	"	"
1634		"	"	"	"

These emulsions, with the exception of that from the rib marrow, were very rich in tissue, the object being to inject as large a dose as possible.

Guinea-Pigs inoculated with various Tissues of

Calf 881 (H. 26. "K.M.").

[Killed 8 days after injection.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose.	Duration of Life in Days.	Killed or Died.	Result.
1635	Blood from Heart - -	11·0 cc.	41	Killed	6 tubercles in Spleen.
1636	E. of Rib Marrow - -	5·0 cc.	"	"	T.
1637		"	"	"	No T.
1638	E. of Popliteal Gland - -	10·0 cc.	"	"	G.T.
1639	E. of Thoracic Gland - -	4·0 cc.	"	"	"
1640	E. of Lung - - - -	8·0 cc.	"	"	"
1641		"	"	"	"
1642	E. of Liver - - - -	10·0 cc.	"	"	"
1643		8·0 cc.	"	"	"
1644	E. of Spleen - -	10·0 cc.	"	"	"
1645		8·0 cc.	"	"	"
1646	E. of Kidney - - -	4·0 cc.	"	"	"
1647		5·0 cc.	"	"	"

These emulsions, with the exception of that from the rib marrow, were very rich in tissue, the object being to inject as large a dose as possible.

Guinea-Pigs inoculated with various Tissues of

Calf 869 (H. 26, "K.M.").

[Killed 14 days after injection.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose	Duration of Life, in Days.	Killed or Died.	Result
1648	Blood from Heart and Iliac Vein	2·0 to 3·0 cc.	35	Killed	No T
1649	E. of Rib Marrow - -	6·0 cc.	,,	,,	T.
1650		4·0 cc.	,,	,,	No T.
1651	E. of Spleen - - -	9·0 cc.	,,	,,	G.T.
1652		,,	,,	,,	,,
1653	E. of Liver - - - -	9·0 cc.	,,	,,	,,
1654		,,	,,	,,	,,
1655	E. of Thoracic Gland - -	5·0 cc.	,,	,,	,,
1656	E. of Kidney - - -	9·0 cc.	,,	,,	S.T.
1657		,,	,,	,,	,,
1658	E. of Lung - - - -	6·0 cc.	,,	,,	G.T.
1659		,,	,,	,,	,,
1660	E. of Popliteal Gland - -	6·0 cc.	,,	,,	,,

These emulsions, with the exception of that from the rib marrow, were very rich in tissue, the object being to inject as large a dose as possible.

Guinea-Pigs inoculated with various Tissues of

Calf 871 (H. 26, "K.M.").

[Killed 91 days after inoculation.]

Intraperitoneal Injections.

No. of Guinea-pig.	Material injected.	Dose.	Duration of Life.	Killed or Died.	Result.
1744	Blood from Jugular Vein -	13·0 cc.	A few hours.	Died	—
1745	E. of Rib Marrow - -	6·0 cc.	2 days.	,,	—
1746		6·0 cc.	53 ,,	Killed	Healthy.
1749	E. of Lung - - - -	10·0 cc.	,, ,,	,,	T.
1750		10·0 cc.	,, ,,	,,	,,
1751	E. of Liver - - - -	9·5 cc.	,, ,,	,,	,,
1752		9·5 cc.	,, ,,	,,	,,
1753	E. of Spleen - -	8·0 cc.	,, ,,	,,	S.T.
1754		8·0 cc.	,, ,,	,,	,,
1755	E. of Kidney - - -	6·0 cc.	,, ,,	,,	Healthy.
1756		6·0 cc.	,, ,,	,,	,,

These emulsions, with the exception of that of the rib marrow, were very rich in tissue, the object being to inject as large a dose as possible.

Guinea Pigs inoculated with various Tissues of

Calf 865 (H. 26 "K.M.").

[Killed 93 days after inoculation.]

Intraperitoneal Injections.

No. of Guinea-Pig.	Material Injected.	Dose.	Duration of Life.	Killed or Died.	Result.
1763	Blood from Left Jugular Vein	12·0 cc.	24 hours	Died	—
1764		12·0 cc.	$\frac{1}{6}$ day		—
1765	E. of Liver	10·0 cc.	51 day	Killed	Healthy.
1766		10·0 cc.	,, ,,	,,	,,
1767	E. of Lung	15·0 cc.	9 ,,	Died	No T.
1768		15·0 cc.	51 ,,	Killed	S.T.
1769	E. of Spleen	8·0 cc.	,, ,,	,,	Healthy.
1770		8·0 cc.	,, ,,	,,	,,
1771	E. of Kidney	6·0 cc.	,, ,,	,,	,,
1772		6·0 cc.	,, ,,	,,	,,
1773	E. of Rib Marrow	6·0 cc.	,, ,,	,,	,,
1774		6·0 cc.	,, ,,	,,	,,
1775	E. of Popliteal Gland	10·0 cc.	,, ,,	,,	G.T.
1776		10·0 cc.	50 ,,	Died	,,
1777	E. of Long Thoracic Gland	10·0 cc.	51 ,,	Killed	,,
1778	E. of Bronchial Gland	10·0 cc.	,, ,,	,,	,,
1779	E. of Hepatic Gland	8·0 cc.	,, ,,	,,	,,

These emulsions, with the exception of that of the rib marrow, were very rich in tissue, the object being to inject as large a dose as possible.

From time to time during the course of the work here, guinea-pigs have been injected with emulsions of glands distant from the seat of injection and of organs of bovine animals which have been inoculated with the tubercular viruses of human Group II. Sometimes these have been made for the purpose of elucidating the nature of some suspicious focus; sometimes with the direct object of seeing whether the bacilli were to be found in the spleen or some other part, at others to demonstrate (not always successfully) the non-tubercular origin of intercurrent disease, such, for example, as pneumonia, or carnification of lung following an old blocking of a bronchus.

As these experiments add some additional evidence of passive dissemination to that already brought forward, they are collected together into the following table. As this kind of experiment was not made as a rule, but only for one of the reasons given above, it is probable that the calves from which the tissue emulsions came were not average samples of those injected with viruses non-virulent for bovines, but were on the whole taken from those rather more severely affected than their fellows, or from animals suffering from some other disease of more or less severity.

The frequency with which tubercle bacilli were found in these emulsions of organs then must not be taken as an exact measure of the frequency of passive dissemination in calves which have been subcutaneously injected. These experiments are only referred to here as additional evidence of the fact that such dissemination does occur.

It is a point of some interest that the animals fed with viruses of human Group II. afforded no evidence of passive dissemination though they developed some small amount of tuberculosis in the mesenteric glands. There is not enough evidence of this kind to warrant the drawing of any conclusions, but it would appear that passive dissemination does not occur unless the continuity of tissue is broken, as in subcutaneous injection.

INJECTION OF GUINEA-PIGS WITH TISSUES (OTHER THAN LOCAL LESION AND NEAREST GLANDS) OF CALVES OR HEIFERS WHICH HAD BEEN INJECTED WITH HUMAN VIRUSES OF GROUP II.

I.—Subcutaneous Injections.

No. of Calf or Heifer.	Virus.	No. of Days between injection and killing of Bovine Animal.	Organ or Gland used for injection of Guinea Pig.	Appearance of Organ or Gland at Post-Mortem.	Nos. of Guinea Pigs inoculated.	Result.
Injections of Lung.						
177	H. 8, “S.C.”	54	Lung	Collapsed area of lung. No tubercles seen in the organ	765–766	T.
413	H. 15, “I.W.”	70	,,	Small collapsed area of lung. No tubercles seen in the organ	1348–349	T.
443	H. 11, “E.D.”	72	,,	Right anterior lobe collapsed and carnified. No tubercles seen in organ	1366–367	No T.
737	H. 9, “C.T.”	87	,,	Few calcareous foci found in the organ	598	T.
619	H. 12, “H.N.”	83	,,	Anterior lobes carnified. No tubercles seen in the organ	1513–514	T.
Injections of Liver or Hepatic Glands.						
185	H. 9, “C.T.”	40	Liver	Minute indefinite pale foci seen on surface	761–762	T.
265	H. 17, Sp. B.	107	Hepatic gland	A few calcareous grains in cortex	1048–49	T.
277	,, ,,	89	Liver	One pinhead tubercle only	1087	No T.
			Hepatic gland	Several yellow calcareous foci	1086	T.
Injections of Spleen.						
207	H. 2, Sp. “A”	54	Spleen	Apparently normal	821–822	No T.
331	H. 9, “C.T.”	66	,,	,, ,,	1250–251	T.
367	H. 15, “I.W.”	56	,,	,, ,,	1244–245	T.
157	H. 16, “J.H.”	67	,,	,, ,,	749–750	No T.
265	H. 17, Sp. “B”	107	,,	Two small calcareous tubercles	1046–47	T.
277	H. 17, Sp. “B”	89	,,	Apparently normal	1085	T.
279	H. 21, “G.B.”	91	,,	Three tubercles seen	1114	T.
Injections of Thoracic Glands.						
275	H. 8, “S.C.”	59	Peribronchial gland	Cortex infiltrated with calcareous foci	1131	T.
269	H. 12, “H.N.”	114	Bronchial gland	Apparently normal	1066–67	No. T.
277	H. 17, Sp. “B”	89	Thoracic gland	A few yellow foci in cortex	1088	T.
227	,, ,,	114	,,	Apparently normal	1056	T.
131	H. 18, “T.T.”	139	Bronchial gland	Congested; no tubercles seen	870	T.
279	H. 21, “G.B.”	91	Thoracic gland	Minute calcareous foci in cortex	1115	T.

II.—Feeding Experiments.

No. of Calf or Heifer.	Virus.	No. of Days between injection and killing of Bovine Animal.	Organ or Gland used for injection of Guinea Pig.	Appearance of Organ or Gland at Post Mortem.	Nos. of Guinea Pigs inoculated.	Result.
Injections of Liver.						
51	H. 4 “M.L.”	248	Liver	Three minute pale foci seen	497	No T.
Injections of Spleen.						
51	H. 4 “M.L.”	248	Spleen	Apparently normal	498	No T.
57	H. 6 “U.H.”	146	,,	,, ,,	491–492	No T.
171	H. 17, Sp. “B.”	154	,,	,, ,,	902–903	No T.

EARLY DISSEMINATION OF TUBERCLE BACILLI IN A RAT.

In a rat, which received an intraperitoneal injection of culture from human spleen "H. 54, C.W." on 4-10-05., and which died without ascertained cause thirteen days later, tubercle bacilli were found in large numbers on microscopic examination of the spleen and liver pulp. A few were found also in the lungs, but none in the kidney. There was no lesion visible to the naked eye, except in the omentum which was drawn up and contained opaque yellowish-white collections, which, however, seemed to consist chiefly of the material injected.

Calf 769 (H. 45, "F.M.").

Subcutaneous inoculation on right side of neck of culture derived from human bronchial glands, through Guinea-Pig 1,137.

Dose.—50 milligrammes.

Date of Inoculation.—April 19th, 1905.

Weights :—

April 19th, 1905.—1 cwt. 0 qrs. 5 lbs.
May 6th, 1905.—0 cwt. 3 qrs. 10 lbs.
Total loss of weight :—23 lbs.

Died of diarrhœa, May 6th, 1905 (17 days after inoculation).

Post-mortem Examination.

General Condition.—Very thin.

Seat of Inoculation.—At the seat of inoculation, on the right side of the neck, is a patch, 2½in. x 2in., composed of inflammatory tissue, soft and commencing to break down.

Right Prescapular Gland	Are slightly swollen, but show no sign of tubercle.
Right Prepectoral Gland	

Thoracic Glands.—Show no sign of tubercle.

Lungs.—Several dark points are seen, one of which only has a minute opaque centre. Of the rest, two, reddish and slightly raised, are situated on the visceral pleura. The lungs show superficial congestion about their bases.

Pleura	Normal.
Heart	
Omentum	
Peritoneum	
Liver	
Hepatic Glands	
Spleen	
Kidneys	
Suprarenals	
Tongue	
Pharynx	
Palate	
Tonsils	
Posterior-Pharyngeal Lymphatic Glands	
Submaxillary Lymphatic Glands	
Hyoid Lymphatic Glands	
Subparotid Lymphatic Glands	

Intestines.—The lower part of the small intestine, and the large intestine, are greatly congested, and show patches of bright red colour.

Mesenteric Glands	Normal.
Testes	

Various Lymphatic Glands :—

Left Prescapular	Normal.
Axillary	
Precrural	
Popliteal	
Gluteal	
Ischiatic	
Renal	
Lumbar	
Iliac	
Pubic	

Microscopic Examination.

Yellow Speck from Intestine	No tubercle bacilli.
Grey Firm Specks from Lung (1)	
(2)	
(3)	
(4)	
Scraping from Prescapular Gland	Tubercle bacilli fairly numerous.
Scraping from Prepectoral Gland	
Scraping from Long Thoracic Gland	Tubercle bacilli few.

Comment on Calf 769. H 45. "F.M." May 6th, 1905.

"This calf suffered for some time from, and ultimately succumbed to, diarrhœa. At the post-mortem examination the tubercular disease was found circumscribed as usual when viruses of the non-virulent kind are injected. The new fact revealed by this case was the considerable number of bacilli in the prescapular, prepectoral, and long thoracic glands. The two former were somewhat swollen, but showed no naked eye changes characteristic of tubercle, while the latter was normal in size. These bacilli were found then in a general scraping of the gland, while in ordinary cases, even when tubercular lesions are found in the thoracic glands, tubercle bacilli are limited to the visibly affected areas, and are even so very scanty. There were therefore in this case a much larger number of bacilli in the long thoracic gland than usual, and the question arises whether the tuberculosis was making a more severe progress in this case than usual on account of the intermittent disease, or whether a similar distribution of bacilli occurs in other cases, followed by their subsequent disappearance. The former hypothesis is not supported by other cases, where intercurrent disease has existed, while the latter is in harmony with the finding of minute arrested tubercles in small numbers in some of the animals injected with non-virulent viruses.

Calf 767 (H. 46, "H.W.").

Subcutaneous inoculation on right side of neck of culture derived from Guinea-pig 1444, inoculated with emulsion of tuberculous human knee joint.

Dose.—50 Milligrammes.

Date of Inoculation.—April 28th, 1905.

Weights :—

April 28th, 1905.—1 cwt. 0 qrs. 15 lbs.
May 23rd, 1905.—1 cwt. 0 qrs. 18 lbs.
Total gain of weight : 3 lbs.

Killed.—May 23rd, 1905 (25 days after inoculation), at a time when the febrile reaction was just over, and the enlargement of the prescapular gland probably at its height

Post-mortem Examination.

General Condition.—Good.

Seat of Inoculation.—The tumour at the seat of inoculation, on the right side of the neck, is large, flat, and fluctuating. It measures 6 in. x 3½ in. It is closely attached to the subjacent muscle, and slightly infiltrates the skin.

On section it is composed of caseo-necrotic tissue, and has large ragged cavity in its centre, full of caseous flakes suspended in a serous fluid.

Right Prescapular Gland.—Measures 3 in. x 1½ in. x 1¾ in. It is firm and elastic, and consists partly of translucent grey tissue, and partly of a caseating network. Its capsule is much thickened, and it is very adherent to the surrounding tissues.

Right Prepectoral Gland.—The size of a large pea, is firmer than normal, and in part of its cortex numerous yellow points could be faintly seen.

Thoracic Glands.—Normal.

Lungs.—On the surface of the lungs rather numerous minute grey translucent points can be seen. Some of these have a reddish tinge, and it is difficult to distinguish them from the minute hæmorrhages, which are of frequent occurrence. Some are undoubtedly tubercles, of the kind frequently found in these cases, for they can be picked out on the point of a knife. Scarcely any trace of opacity can be seen in the centres of any of them, and when crushed they do not break under the knife.

Pleura	Normal.
Heart	
Omentum	
Peritoneum	

Liver.—One or two pale specks on the surface is the nearest suggestion of tubercles that can be seen.

Hepatic Glands	Normal.
Spleen	

Kidneys.—A pale speck, similar to those seen in the liver, was seen in each kidney.

Suprarenals	Normal.
Tongue	
Pharynx	
Palate	
Tonsils	
Posterior Pharyngeal Lymphatic Glands	
Submaxillary Lymphatic Glands	
Hyoid Lymphatic Glands	
Subparotid Lymphatic Glands	
Intestines	
Mesenteric Glands	
Eyes	
Testes	

Various Lymphatic Glands:—

Left Prescapular	Normal.
Axillary	
Precrural	
Popliteal	
Gluteal	
Ischiatic	
Renal	
Lumbar	
Iliac	
Pubic	

Microscopic Examination.

Focus from Lung.—No tubercle bacilli.

Comment on Calf 767.

In some respects the local lesion and nearest gland resembled those found in cases of acute general tuberculosis, but the muscle, though adherent, was not infiltrated, as it invariably is in these latter cases, and the central cavity was much larger, showing a greater tendency to softening and breaking down. The lesions in the lungs were no more than are frequently seen in calves inoculated with non-virulent viruses, unless indeed there were rather more of them. They were very small, and, unlike those in calves killed ninety days after inoculation, they were not calcified.

In choosing this calf, one was influenced by the longer rise of temperature and by the larger size of the prescapular gland. But really there was little to choose between the animals, and the other had lost two pounds in weight, while the one killed had gained three. The remaining calf therefore is a fair control, and it must not be thought that the one killed was any more likely to develop general tuberculosis than the other.

Calf 777 (H. 46, "H.W.").

Subcutaneous inoculation on right side of neck of culture derived from Guinea-pig 1444, inoculated with emulsion of tuberculous human knee joint.

Dose.—50 milligrammes.

Date of Inoculation.—April 28th, 1905.

Weights,—
April 28th, 1905.—1 cwt. 0 qrs. 4 lbs.
July 27th, 1905.—1 cwt. 0 qrs. 6 lbs.
Total gain of weight.—2 lbs.

Killed when in good health, July 27th, 1905. (90 days after inoculation.)

Post-mortem Examination.

General Condition.—Good.

At the Seat of Inoculation on the right side of the neck is a prominent fluctuating swelling measuring 4 x 2½ x 2in., and weighing 6½ ozs.

It consists of a fibrous walled cavity containing softened caseous débris.

There has been no external discharge.

Right Prescapular Gland is moderately enlarged, and contains two large fibro-calcareous nodules, the larger of which is already considerably softened.

Right Prepectoral Gland.—Not enlarged, is normal on section.

Lungs.—The lungs show several small areas of collapse, but are otherwise normal.

Thoracic Glands.—are not enlarged and are normal in structure.

Heart	Normal.
Pleura	
Omentum and Peritoneum	
Liver	
Hepatic Glands	
Spleen	
Kidneys	
Suprarenals	
Tongue	
Pharynx	
Palate	
Tonsils	
Posterior-Pharyngeal, Submaxillary, Hyoid and Subparotid Lymphatic Glands	

Mesenteric Glands.—The hooked mesenteric gland, which lies at the angle of the mesentery, contains many minute points visible throughout its capsule. These when picked out on the point of a knife and crushed between coverslips, are found to be slightly gritty. A few neighbouring glands also have some of these foci.

Small Intestine.—In the small intestine, about 1½ yards from the ileo-caecal valve, the part corresponding with the gland most affected, are seen a small number of whitish spots, quite superficial and easily scraped off with a knife, unlike tubercles as usually seen. The large intestine is normal.

Testes.—Normal.

Various Lymphatic Glands:—

Axillary and Prescapular (left)	Normal.
Precrural and Popliteal	
Gluteal and Ischiatic	
Renal	
Lumbar	
Iliac	
Pubic	

MICROSCOPIC EXAMINATION.

Foci from Mesenteric Gland.—Tubercle bacilli.

Foci from Small Intestine.—No tubercle bacilli.

COMMENT ON CALF 777.

The minimal lesions usual when non-virulent viruses are injected were found in this animal.

This animal is the fellow to Calf 767, which was killed 25 days after inoculation in order that the various organs and tissues should be examined for tubercle bacilli. It therefore forms the control to the experiment just mentioned.

Calf 873 (H. 26, "K.M.").

SUBCUTANEOUS inoculation, on right side of neck, of culture derived from human kidney, through guinea-pig 1326.

DATE OF INOCULATION.—July 25th, 1905.

DOSE.—50 milligrammes.

KILLED.—July 28th, 1905 (3 days after inoculation), in order to determine the distribution of bacilli at this period.

POST-MORTEM EXAMINATION.

General Condition.—Good.

Seat of Inoculation.—At the seat of inoculation, on the right side of the neck, is a small soft swelling. On section the tissues seem to be a little oedematous, and the subcutaneous tissue thickened and firmer than normal.

Right Prescapular Gland.—Is somewhat swollen and oedematous.

Thoracic Glands	Normal.
Lungs	
Heart	
Pleura	
Peritoneum	
Liver	
Hepatic Glands	
Spleen	
Kidneys	
Suprarenals	
Pharynx	
Tongue	
Tonsils	
Intestines	
Mesenteric Glands	
Testes	

All lymphatic glands were examined, and found normal.

Calf 881 (H. 26, "K.M.").

SUBCUTANEOUS inoculation on right side of neck of Culture derived from human kidney, through Guinea-pig 1326.

DATE OF INOCULATION. –July 25th, 1905.

DOSE.—50 milligrammes.

Weights.

July 25th, 1905.—0 cwt., 3 qrs., 15 lbs.

August 2nd, 1905.—0 cwt., 3 qrs., 26 lbs.

Total gain of weight.—11 lbs.

Rate of gain per week.—10 lbs.

KILLED when very well, August 2nd, 1905 (8 days after inoculation), in order to determine the distribution of bacilli at this period.

POST-MORTEM EXAMINATION.

General Condition.—Good.

Seat of Inoculation.—At the seat of inoculation, on the right side of the neck, is a small swelling $2\frac{1}{2}'' \times 2''$.

Section shows a reddish grey and rather firm tissue under the skin, about half an inch thick, infiltrated with spots of the size of shot, of softened caseous matter resembling muco-pus.

Right Prescapular Gland.—Is somewhat swollen, but shows no sign of tubercle.

Lungs	Normal in appearance.
Thoracic Gands	
Heart	
Pleura	
Peritoneum	
Liver	
Hepatic Glands	
Kidneys	
Suprarenals	
Spleen	
Tongue	
Pharynx	
Palate	
Tonsils	
Posterior-Pharyngeal Glands	
Submaxillary Glands	
Hyoid Glands	
Subparotid Glands	
Intestines	
Mesenteric Glands	
Mamma	
Axillary Glands	
Prescapular Gland (Left)	
Precrural Glands	
Popliteal Glands	
Gluteal Glands	
Ischiatic Glands	
Renal Glands	
Lumbar Glands	
Iliac Glands	

MICROSCOPIC EXAMINATION.

No Microscopic Examination was made.

Calf 869 (H. 26, "K.M.").

SUBCUTANEOUS inoculation on right side of neck of Culture derived from human kidney, through Guinea-pig 1326.

DATE OF INOCULATION.—July 25th, 1905.

DOSE.—50 Milligrammes.

Weights.

July 25th, 1905.—0 cwt. 3 qrs. 0 lbs.
August 8th, 1905.—0 cwt. 3 qrs. 9 lbs.
Total gain of weight.—9 lbs.

KILLED—When very well, in order to determine the distribution of bacilli, August 8, 1905 (14 days after inoculation).

POST-MORTEM EXAMINATION.

Seat of Inoculation.—At the seat of inoculation is a tumour, measuring 4″ x 3″ x 1¼″. At its centre is an irregular cavity, containing muco-purulent material, around which is dense oedematous tissue. The subjacent muscle is not infiltrated, but the skin over the tumour is distinctly altered in appearance.

Right Prescapular Gland.—Is enlarged (long axis three and a quarter inches), firm, elastic, and oedematous. There is no appearance characteristic of tubercle.

Prepectoral Gland.—Is the size of a large pea. It is very similar to the prescapular, but of a yellower colour.

Lung *Thoracic Glands* *Liver* *Portal Glands*	Showed nothing abnormal
Spleen *Kidneys* *Suprarenal* *Testes* *Intestine* *Mesenteric Glands* *Pharynx* *Posterior-Pharyngeal Glands* *All Peripheral Glands*	Normal.

MICROSCOPIC EXAMINATION.

Emulsion of Liver *Emulsion of Lung* *Emulsion of Kidney*	No tubercle bacilli seen.

Calf 871 (H. 26. "K.M.").

SUBCUTANEOUS inoculation, on right side of neck, of Culture derived from human kidney, through Guinea-pig 1326.

DATE OF INOCULATION.—July 25, 1905.

DOSE.—50 Milligrammes.

Weights.

July 25th, 1905.—0 cwt. 3 qrs. 19 lbs.
October 24th, 1905.—1 cwt. 0 qrs. 18 lbs.
Total gain of weight.—27 lbs.
Rate of gain per week.—2 lbs.

KILLED.—When well, October 24th, 1905 (91 days after inoculation).

POST-MORTEM EXAMINATION.

Seat of Inoculation.—At the seat of inoculation is a large, rounded, fluctuating swelling, consisting of a fibrous wall and fluid contents, with caseo-necrotic masses of various sizes. At the posterior corner are some small calcareous nodules. It measures 3¾in. x 2⅛in. x 3 in., and weighs 9 ozs.

Prescapular Gland.—Measured 2⅛in. x 1⅝in. x 1in., and weighs 2 ozs. It was almost entirely occupied by a large and very calcareous mass, but a portion of the soft brown gland substance still remained.

Prepectoral Gland.—Was as large as a small cherry, and was also calcareous.

Bronchial Glands. and Other Thoracic Glands	Were not enlarged. Here and there a small yellow calcareous focus could be found.

Lungs.—Looked quite healthy, but on close inspection a good many minute translucent glassy tubercles could be seen. The largest of these was only one millimetre in diameter. Few of them had any central opacity. In addition two rather large shot-like tubercles were seen.

Liver.—Normal.

Hepatic Glands.—Showed one or two little doubtful points.

Spleen.—Normal.

Kidneys.—Normal, except for one or two very minute pale specks.

Suprarenals.—Each contained one or two small tubercles.

Testes *Tongue* *Pharynx* *Tonsils* *Larynx*	Normal.

Intestines.—Normal, except for some pultaceous yellow points (with no tubercle bacilli).

Left Posterior Pharyngeal Gland.—Had two or three small calcareous grains.

Right Submaxillary Gland *Right Post-Pharyngeal Gland* *Hyoid Glands* *Subparotid Glands*	Normal.

Mesenteric Glands.—Almost without exception contained little yellow calcareous grains.

The following peripheral glands contained a few little yellow calcareous grains :—

Precrural.
Popliteal.
Gluteal.
Ischiatic.

The following were normal :—

Iliac.
Axillary.
Renal.
Lumbar.

MICROSCOPIC EXAMINATION.

Calcareous Grains from the Popliteal Gland.—Tubercle bacilli few.

Calcareous Grains from the Long Thoracic Gland.—Tubercle bacilli. (Two seen.)

Glassy Tubercles from Lungs *Larger Tubercle from Lung*	No tubercle bacilli.

Large and small Tubercles from Lung (mixed).—Tubercle bacilli few.

Emulsion of Spleen *Emulsion of Liver* *Emulsion of Rib Marrow* *Yellow Pultaceous Matter from Intestine*	No tubercle bacilli.

COMMENT ON CALF 871.

The animal developed a very large swelling and gland, and a fortnight after injection was obviously ill, with rapid respiration and staring coat. There was the usual rise of temperature, lasting two or three weeks. Later on it became thin, but afterwards picked up, and gained 27 lbs. It had been quite well for a month or more before it was killed.

The lesions seen were like those often before found in animals injected with non-virulent viruses. Thus there are numerous parallels to the lung lesions, and tubercles in the suprarenals have been fairly common, *e.g.*, 135, H.12, "H.N.," and many others.

To find a parallel to so wide a distribution of calcareous grains in the peripheral lymphatic glands one must go to 619, H.12, "H.N." 625, H.23, "J.P." The peculiarity of this calf is that it combines so many of these occasional lesions.

It is odd that while many peripheral glands were more or less affected, the renal and lumbar, which with virulent bacilli are usually more severely affected than the others, in this case escaped.

Calf 865 (H. 26. "K.M.").

SUBCUTANEOUS inoculation, on right side of neck, of Culture derived from human kidney, through guinea-pig 1326.

DATE OF INOCULATION.—July 25th, 1905.

DOSE.—50 milligrammes.

Weights.
July 25th, 1905.—0 cwt. 3 qrs. 12 lbs.
October 26th, 1905.—1 cwt. 1 qr. 9 lbs.
Total gain of weight.—1 qr. 25 lbs.
Rate of gain per week.—4 lbs.

KILLED.—When well, October 26th, 1905 (93 days after inoculation).

POST-MORTEM EXAMINATION.

General Condition.—Good.

Seat of Inoculation.—At the seat of inoculation, on the right side of the neck, is a little opening in the skin, and beneath it is a pinkish-brown patch of dense brownish tissue packed with little calcareous grains.

Right Prescapular Gland.—Is a little enlarged, two and a quarter inches in length. It contains some large calcareous nodules, and one in process of softening.

Right Prepectoral Gland.—Normal.

Lungs.—Normal. (Here and there one could see some little grey specks, more minute by far than those which have frequently been described.)

Bronchial Glands
Thoracic Glands
Peribronchial Glands
Normal.

Pleura.—On the costal lining is a little pedunculated body the size of a grape seed. (No tubercle bacilli in it).

Heart
Peritoneum
Liver
Hepatic Glands
Spleen
Normal.

Kidneys
Suprarenals
Testes
Pancreas
Thyroid
Tongue
Pharynx
Larynx
Posterior Pharyngeal Lymphatic Glands
Submaxillary Lymphatic Glands
Hyoid Lymphatic Glands
Subparotid Lymphatic Glands
Intestines
Normal.

Mesenteric Glands.—Here and there in these glands was a little yellow calcareous focus.

Various Lymphatic Glands:—
Left Prescapular
Axillary
Precrural
Popliteal
Gluteal
Ischiatic
Renal
Lumbar
Iliac
Pubic
Normal.

MICROSCOPIC EXAMINATION.

Calcareous Foci from Mesenteric Glands (All that could be found).—Tubercle bacilli.

Pedunculated Body from Pleura.—No tubercle bacilli.

COMMENT ON CALF 865.

Minimal lesions after 50 milligrammes of a feebly virulent virus.

The little grey points in the lungs would not have been thought worthy of notice if it were not considered that they might possibly be the legacy of the bacilli which escaped into the lung.

REPORT ON THE DISSEMINATION OF TUBERCLE BACILLI IN DIFFERENT SPECIES OF ANIMALS AFTER SUBCUTANEOUS INOCULATION.

Dr. A. STANLEY GRIFFITH and Dr. F. GRIFFITH.

ROYAL COMMISSION ON TUBERCULOSIS.

ON THE DISSEMINATION OF TUBERCLE BACILLI IN DIFFERENT SPECIES OF ANIMALS AFTER SUBCUTANEOUS INOCULATION.

It has been shown by Dr. Cobbett that slightly virulent tubercle bacilli are rapidly distributed throughout the body of a calf after subcutaneous inoculation, and can be demonstrated in the tissues by inoculating guinea-pigs.

The object of these experiments has been to ascertain whether a similar distribution takes place after subcutaneous inoculation of highly virulent tubercle bacilli, not only in the calf but also in other species of animals.

In five experiments bacilli of low bovine virulence were used.

Observations have been made on eight different species of animals, namely, the monkey, calf, pig, cat, rabbit, guinea-pig, rat and fowl.

The following table gives in brief the dose of culture and the duration of life in the case of each animal used in the investigation.

Species.	Number of Animals.	Dose of Culture.	Duration of Life.
Monkey - -	1	20 mg.	24 hours.
Calf - - -	1	44 mg.	24 hours.
Pig - - - -	5	20 mg.	1, 3, 5, 8, and 14 days.
Cat - - - -	3	10 mg.	24 hours.
Rabbit - - -	3	20 (1) and 10 mg. (2)	24 (2) and 48 (1) hours.
Guinea-Pig - -	3	10 mg.	24 (2) and 48 (1) hours.
Rat - - - -	2	10 mg.	24 and 48 hours.
Chicken - - -	1	10 mg.	24 hours.

The subcutaneous tissues of the groin, back, or ear were chosen as the site of inoculation in order to obviate, during the removal of the organs, any possible chance of contamination by the culture inoculated.

The animals were killed by chloroform, and in every case blood was immediately withdrawn from the right ventricle by means of a glass pipette. A small quantity was distributed over the surface of each culture tube, while 2 cc., mixed with an equal amount of citrate of soda solution, were reserved for inoculating into a guinea-pig. The portions of the various organs from which cultures were made and guinea-pigs inoculated were removed with aseptic precautions.

The egg medium, which has been found the most reliable of all media for the production of primary cultures, was used exclusively, and the tubes were incubated for periods of two months.

The experiments show that tubercle bacilli are distributed over the body of an animal within 24 hours after subcutaneous inoculation, and this dissemination occurs in every species inoculated, whether the bacilli are virulent or only slightly virulent for that species.

That no inconsiderable number of bacilli escapes into the circulation is shown by the ease with which cultures were in all cases obtained from the spleen and by the production of tuberculosis in every guinea-pig inoculated with blood.

In the series of pigs killed at intervals the number of bacilli in the blood was greatest 24 hours after inoculation, and appeared gradually to diminish subsequently.

Seeing that in all species of animals the blood stream plays so important a part in the immediate distribution of tubercle bacilli over the body, the variations in the anatomical distribution of the disease which have been observed in different species after subcutaneous inoculation can best be explained on the hypothesis that different tissues vary in their susceptibility to tuberculosis.

It is interesting to observe in the case of the chicken the rapidity with which bacilli, which are incapable of setting up tuberculosis in that animal, are distributed over the body.

A. Stanley Griffith.

F. Griffith.

RHESUS MONKEY 150.

Inoculated subcutaneously between the shoulders on June 7, 1906, with 20 mg. of culture from the original material of Virus XXVI.

Killed June 8, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material Used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood	3	None.	2,071	Died 8 days	No tuberculosis.
			2,072	Killed 33 days	Discrete tubercles in omentum ; slight tuberculosis of spleen and of most of abdominal lymphatic glands.
Spleen	3	Numerous colonies on all the tubes.	2,073	Killed 33 days	General tuberculosis.
			2,074	Died 19 days	Slight tuberculosis.
Marrow	2	2	—	—	—

CALF 438.

Inoculated subcutaneously on the left side of the neck on June 22, 1906, with 44 mg. of culture of Viruses XXIX. and XXX.

Killed June 23, 1906, 24 hours after inoculation.

P.M. :—Organs and glands perfectly normal to the naked eye.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood	3	None	2,097	Killed 27 days	General tuberculosis; scattered discrete tubercles in omentum.
			2,098	Killed 27 days	Slight tuberculosis of omentum, spleen, liver and of several lymphatic glands.
Lung	—	–	2,099	Killed 27 days	General tuberculosis.
			2,100	Killed 27 days	General tuberculosis.
Spleen	3	13	2,101	Killed 27 days	General tuberculosis.
			2,102	Killed 27 days	No tuberculosis.
Liver	2	4	2,105	Killed 27 days	General tuberculosis.
Kidney	—	—	2,106	Killed 27 days	Slight tuberculosis of omentum, liver and spleen and of several lymphatic glands.
Popliteal gland	—	—	2,108	Killed 27 days	
Marrow	2	1	2,107	Killed 27 days	
Muscle	–	—	2,103	Killed 27 days	One tubercle in omentum; pyloric gland partly caseous ; portal gland slightly affected.
			2,104	Killed 27 days	No tuberculosis.
Prescapular gland	2	1	—	—	—

PIG 144.

Inoculated subcutaneously under the skin of the ear on May 4, 1906, with 20 mg. of culture from Virus B. V.

Killed, May 5, 1906, 24 hours after inoculation.

P.M.—There was a small patch of necrosis in the subcutaneous tissues of the ear and no sign of disease elsewhere

DISTRIBUTION OF BACILLI.

Material Used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	3	1,988	Killed 32 days	General tuberculosis.
Lung - -	—	—	1,991	Killed 32 days	Slight general tuberculosis; discrete tubercles in omentum.
Spleen - -	3	5	1,993	Died 32 days	General tuberculosis; discrete tubercles in omentum.
Liver - -	—	—	1,994	Died 12 days	Subacute infection; no tuberculosis.
Muscle - -	—	—	1,989	Killed 32 days	No tuberculosis.
			1,990	Killed 32 days	No tuberculosis.
Marrow - -	1	None	—	—	—

PIG 146.

Inoculated subcutaneously under the skin of the ear on May 2, 1906, with 20 mg. of culture from Virus B.V.

Killed, May 5, 1906; 3 days after inoculation.

P.M.—In the subcutaneous tissues of the ear there was a yellow patch of necrosis, larger than in Pig 144, undergoing softening. The nearest lymphatic gland was slightly enlarged. There was no sign of disease in any other part of the body.

DISTRIBUTION OF BACILLI.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	1,995	Died, 23 days	Two or three minute transparent tubercles in omentum; pseudotuberculosis of spleen and liver. Microscopical. ? T.B. in omentum; none in liver or spleen.
Lung - -	—	—	1,998	Killed, 39 days	General tuberculosis.
			1,999	Died, 39 days	General tuberculosis.
Spleen -	2	3	2,000	Died, 24 days	Numerous discrete tubercles in omentum; tuberculosis of spleen and liver and many glands. T.B. found in a splenic gland.
Liver - -	—	—	2,001	Died, 25 days	General tuberculosis. T.B. in the pyloric gland.
Muscle - -	—	—	1,996	Died, 39 days	Caseous nodule in muscle wall; a few tubercles in omentum and on peritoneum; slight tuberculosis of spleen, liver and lungs.
			1,997	Died, 24 days	The omentum contained four translucent miliary tubercles; no tuberculosis elsewhere. T.B. found in omental tubercle.
Marrow - -	—	1	—	—	—

PIG 148.

Inoculated subcutaneously under the skin of the ear on April 30, 1906, with 20 mg. of culture from Virus B. V.
Killed, May 5, 1906, 5 days after inoculation.

P.M.—In the subcutaneous tissues of the ear there was a small swelling composed of yellow necrotic breaking down substance. The nearest gland was enlarged and œdematous. There was no sign of disease elsewhere.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	2,002	Died, 41 days	Small caseous nodule in muscle wall ; nearest glands tuberculous ; two tubercles in the omentum and one on the mesentery.
Lung - - -	—	—	2,005	Died, 28 days	General tuberculosis.
			2,006	Died, 26 days	General tuberculosis.
Spleen - -	3	2	2,007	Died, 38 days	General tuberculosis ; discrete caseous nodules in the omentum.
Liver - -	—	—	2,008	Died, 29 days	General tuberculosis ; the omentum contained about a dozen miliary tubercles.
Muscle - -	—	—	2,003	Killed, 41 days	Caseous nodule in lung ; bronchial glands greatly enlarged and partly caseous ; spleen enlarged, beset with miliary tubercles ; portal gland caseous ; no disease of omentum.
			2,004	Died, 26 days	No tuberculosis.
Marrow - -	1	None	—	—	—

PIG 142.

Inoculated subcutaneously under the skin of the ear on April 28, 1906, with 20 mg. of culture from Virus B. V.
Killed May 6, 1906, 8 days after inoculation.]

P.M. :—Caseo-necrotic local tumour ; the glands at the root of the ear were enlarged and showed on section very early streaks of necrosis. The lungs showed fairly numerous minute grey tubercles, the largest about 0·5 mm. The liver showed under the capsule a few minute grey points. Other organs and glands appeared normal.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	2,009	Died 12 days	One grey tubercle in omentum ; pyloric gland slightly enlarged ; no tubercle bacilli found.
Lung - -	—	—	2,012	Died 20 days	Early general tuberculosis.
			2,013	Died 20 days	General tuberculosis.
Spleen - -	3	13	2,014	Died 31 days	General tuberculosis.
Liver - -	1	Numerous	2,015	Died 12 days	No naked eye evidence of tuberculosis.
Left precrural gland - -	—	—	2,016	Died 41 days	General tuberculosis.
Muscle - -	—	—	2,010	Died 49 days	Omentum normal ; small pea-sized caseous nodule in lung ; bronchial glands enlarged, firm and partly caseous ; tubercles in spleen.
			2,011	Killed 49 days	Two minute grey foci in the omentum ; caseous nodule in the lung ; bronchial glands enlarged and partly caseous ; tubercles in the spleen.
Marrow - -	1	None	—	—	—

PIG 150.

Inoculated subcutaneously under the skin of the ear on April 25, 1906, with 20 mg. of culture from Virus B. V.

Killed, May 9, 1906, 14 days after inoculation.

P.M.—In the subcutaneous tissues of the ear there was a caseo-necrotic tumour with a cavity in the centre communicating externally through a small opening. At the base of the ear near the angle of the jaw there was a large firm gland which on section showed a yellowish or yellowish-white necrotic network in a matrix of grey translucent tissue ; two neighbouring glands, smaller in size, were similarly affected, but in a less advanced stage of necrosis. The lungs were closely beset with grey tubercles, varying a little in size, the largest not quite 1 mm. in diameter ; a good many of the larger tubercles had minute central opacities. The liver substance contained sparsely scattered grey tubercles, the largest 0·5 mm. in diameter ; there was also one tubercle, a little over 1 mm. in diameter, with a yellow centre. The kidneys contained fairly numerous greyish-white tubercles, the largest 1 mm. in diameter. A portal gland showed a few greyish foci. Other organs and glands appeared normal.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	3	None	2,017	Killed, 34 days	No tuberculosis.
Lung - -	—	—	2,020 2,021	Died, 33 days Died, 32 days	General tuberculosis ; omentum much thickened ; tubercles on peritoneum. General tuberculosis ; omentum much thickened ; numerous tubercles on peritoneum.
Spleen - -	3	1	2,022	Died, 27 days	General tuberculosis.
Liver - -	1	3	2,023	Died, 32 days	General tuberculosis.
Left precrural gland.	—	—	2,324	Died. 32 days	Tuberculosis of omentum (slight), spleen, liver, sternal glands, and most of the abdominal lymphatic glands.
Muscle - -	—	—	2,018 2,019	Died, 25 days Killed, 34 days	One ventral mediastinal gland was caseous (T.B.). There was no disease elsewhere. No tuberculosis.
Marrow - -	2	None	—	—	—

CAT 70.

Inoculated subcutaneously in the left groin, on April 26, 1906, with 10 mg. of culture of Virus B. V.

Killed April 27, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	8	1,973	Died, 24 days	Slight tuberculosis.
Spleen - -	2	1	—	—	—
Marrow - -	1	None	—	—	—

CAT 72.

Inoculated subcutaneously between the shoulders on May 14, 1906, with 10 mg. of culture from a slightly virulent virus.

Killed, May 15, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	2,029	Killed, 30 days	Slight tuberculosis.
Spleen - -	2	Numerous	—	—	—
Lung - -	2	None	—	—	—

KITTEN 90.

Inoculated subcutaneously in the left groin on June 6, 1906, with 10 mg. of culture derived from the original material of Virus B. XXVI.

Killed, June 7, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally.)		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	3	—	2,068	Killed 34 days	Slight tuberculosis, tubercle bacilli found in an omental tubercle.
			2,069	Died 19 days	There was one tubercle in the omentum, in which tubercle bacilli were found, and no sign of disease elsewhere.
Spleen - -	2	20	2,070	Killed 34 days	Slight general tuberculosis.
Marrow - -	2	None	—	—	—

Inguinal glands: A smear preparation from an inguinal gland of the kitten showed numerous tubercle bacilli.

RABBIT 926.

Inoculated subcutaneously between the shoulders on June 26, 1906, with 20 mg. of culture of a slightly virulent virus.

Died, June 27, 1906, in less than 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pig Inoculated.		
	Number of Tubes sown.	Number of Colonies observed.	Number.	Duration of Life.	Result.
Spleen - -	3	3	2,117	Died, 42 days.	General tuberculosis.

RABBIT 832.

Inoculated subcutaneously in the left groin on April 25, 1906, with 10 mg. of culture of Virus B. V.

Killed, April 26, 1906, 24 hours after inoculation.

DISTRIBUTION OF BACILLI.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	1,972	Killed, 28 days	There was a caseous nodule in the muscle wall and two miliary tubercles in the omentum ; one iliac gland was enlarged but not opaque ; there was no disease elsewhere. T.B. found in nodule in muscle wall.
Spleen - -	1	Contaminated	—	—	—
Marrow - -	1	None	—	—	—

RABBIT 809.

Inoculated subcutaneously in the left groin on March 22, 1906, with 10 mg. of culture of a slightly virulent virus.

Killed, March 24, 1906, 48 hours after inoculation.

DISTRIBUTION OF BACILLI.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	1,953	Killed, 34 days	General tuberculosis.
Spleen - -	1	6	—	—	—
Marrow - -	1	None	—	—	—

GUINEA-PIG 1970.

Inoculated subcutaneously in the left groin, on April 25, 1906, with 10 mg. of culture of Virus B. V.

Killed, April 26, 1906, 24 hours after inoculation.

DISTRIBUTION OF BACILLI.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	1,971	Killed, 28 days	Slight tuberculosis.
Spleen - -	2	1	—	—	—
Marrow - -	1	Contaminated	—	—	—

GUINEA-PIG 1948.

Inoculated subcutaneously in the left groin on March 22, 1906, with 10 mg. of culture from a slightly virulent virus.

Killed, March 24, 1906, 48 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood -	2	None.	1,952	Killed 34 days	General tuberculosis.
Spleen -	1	20	—	—	—
Marrow - -	1	2	—	—	—

GUINEA PIG 1949.

Inoculated subcutaneously in the left groin on March 22, 1906, with 10 mg. of culture from a slightly virulent virus.

Killed, March 23, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	None	1,950	Killed, 35 days	Slight general tuberculosis.
Spleen - -	1	2	—	—	—
Marrow - -	1	Contaminated	—	—	—

RAT 89.

Inoculated subcutaneously in the thigh on December 12, 1905, with 5·0 mg. of culture from Virus B. XVIII.

Killed December 13, 1906, 24 hours after inoculation.

Material used.	Cultures.		Guinea-Pig Inoculated (Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	–	None	1,800	K. 22 days	Slight tuberculosis.
Spleen - -	1	10	—	—	—
Marrow - -	–	None	– –	—	—

RAT 90.

Inoculated subcutaneously in the thigh on December 12, 1905, with 5·0 mg. of culture from Virus B. XVIII. Killed, December 14, 1906, 48 hours after inoculation.

Material used.	Cultures.		Guinea-Pig Inoculated (Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	1	1,805	D. 14 days	No tuberculosis.
Spleen - -	1	46	—	—	—
Marrow - -	1	None	—	—	—

CHICKEN 52.

Inoculated subcutaneously in the left groin on May 2, 1906, with 10 mg. of culture from Virus B. V. Killed, May 3, 1906, 24 hours after inoculation.

Distribution of Bacilli.

Material used.	Cultures.		Guinea-Pigs Inoculated (all Intraperitoneally).		
	Number of Tubes sown.	Number of Colonies observed.	Number of Guinea-Pig.	Duration of Life.	Result.
Blood - -	2	7	1,987	Killed, 35 days	Slight tuberculosis.
Spleen - -	2	13	—	—	—
Liver - -	1	Numerous	—	—	—
Marrow -	1	None	—	—	—

REPORT ON THE INFLUENCE OF INDIVIDUAL SUSCEPTIBILITY AND OF DOSE ON THE SEVERITY OF AN EXPERIMENTAL INFECTION BY TUBERCLE BACILLI OF HUMAN ORIGIN.

Dr. L. COBBETT.

REPORT ON THE INFLUENCE ON THE SEVERITY OF AN EXPERIMENTAL INFECTION OF

(1) THE VARIATION IN THE CAPACITY FOR RESISTANCE TO TUBERCULOSIS OF DIFFERENT INDIVIDUAL ANIMALS, AND

(2) THE NUMBER OF TUBERCLE BACILLI INJECTED.

The object of the majority of the injections made here into various kinds of animals has been to ascertain as exactly as possible the comparative virulence of different tubercular viruses. Before exact conclusions could be drawn from these experiments, it was necessary to know what effect upon the result might be caused by differences in (1) the capacity for resistance of different individual animals, and (2) the quantity of tubercle bacilli injected. It also became of importance to know the smallest dose which could be used without these differences in capacity for resistance having a dominating influence upon the result.

(1) The Variation in the Capacity for Resisting Tuberculosis among Individuals of the Same Species of Animal.

(a) *Influence of Age.*

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c.

From some of the many experiments carried out at this farm evidence may be gathered confirmatory of the generally received opinion that among the individuals of a given species the capacity for resistance to tuberculosis increases with age. There can, I think, be no doubt that among bovines, calves are distinctly more susceptible than adults. Of this there is plenty of evidence. Moreover, younger calves are more susceptible than older ones. As a rule when two calves were given the same injection they were approximately of the same age and weight. When occasionally this was not so, the younger animal became more rapidly and severely affected. As examples of this one may refer to Calves 113 and 35 [H10.BS], one of which was nearly double as large as the other when injected, and became very much less severely affected with tuberculosis. Also the two calves, 531 and 555, may be compared with the young bull, 611 [H17. Sp. B], Calf 563 with the young bull, 525 [H 7.C.M.], and Calf 387 with Heifer 249 [H10 B.S.] There are no examples of an older animal being more severely affected than a younger one which received the same dose.

(b) *The Influence of Sex. Pregnancy and Lactation.*

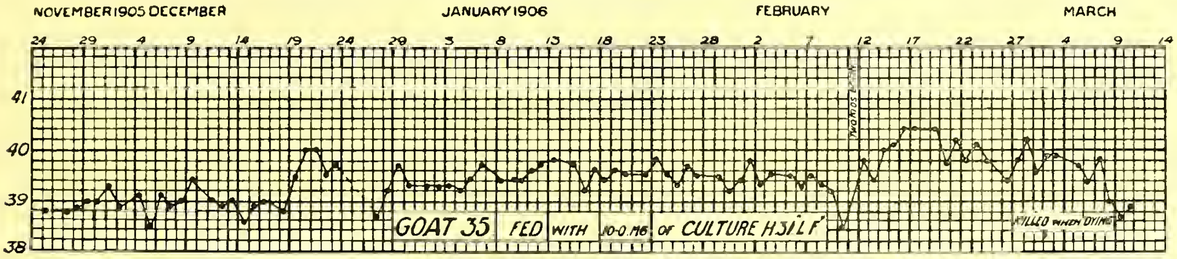
The influence of sex, on the susceptibility to tuberculosis.

Among calves no difference was observed in the capacity of the two sexes to resist tuberculosis.

The influence of pregnancy and the puerperium.

A goat, No.35, which was pregnant when fed with a virus of the human Group I, developed a progressing tuberculosis, and was in poor condition at parturition, 79 days after infection. Immediately after this it rapidly became worse, and a month later was killed when death was imminent. The temperature chart of this animal shows clearly the somewhat sudden change which took place after parturition. No cause for this change was observed other than parturition, unless indeed it was some affection of the udder owing to the fact that the kids were dead, and so could not take the milk of their mother. But the udder never became full, so that it was not even necessary to milk it by hand, and nothing whatever was noticed to be wrong with it during life. At the post-mortem examination both it and the uterus were found quite healthy.

Chart of Goat 35, shewing rise of temperature after parturition,



The course of the disease in this animal supports the common belief that the resistance to tuberculosis is high during pregnancy and suddenly falls to a lower level after parturition. Unfortunately there was no non-pregnant goat infected at the same time with which to compare this animal.

There is little or no evidence confirmatory or otherwise of this belief to be gathered from the heifers which were pregnant when infected with tuberculosis. Of such there were six. In none of these did the condition of the animal undergo any marked change after parturition during the short period that most of them were allowed to live.

It is difficult to say whether pregnancy conferred any additional resistance on these animals, because in only two instances did non-pregnant animals receive identical injections, and so become available for comparison; and in these, unfortunately, the control animals were of a different breed, being Jerseys, while the heifers in calf were Shorthorns. The facts are as follows.

In the Shorthorn Heifer, No. 233, which calved two months after an experimental infection, and was killed four months later, the tuberculosis was minimal, like that in the non-pregnant Jersey Heifer [No. 247], which received an identical injection. It only differed in that in the former animal it had extended to the mammary gland.

In the next pair the tuberculosis was severe, and it was distinctly less acute in the Shorthorn [No. 235], which was in calf, than in the Jersey [No. 243], which was not. This may, of course, be explained on the hypothesis that pregnancy confers some extra capacity for resistance, but on the other hand there is some reason to think the Jersey is racially more susceptible to tuberculosis than the Shorthorn. In evidence of this belief one uncomplicated experiment only can be quoted, namely, that of the Shorthorn Heifer 231 and the Jersey Heifer 237, both injected subcutaneously with a tissue emulsion containing a small dose of the Virus H10. B.S. Neither animal was in calf. The Shorthorn remained well, and developed minimal scattered lesions only, while the Jersey developed severe tuberculosis.

Racial susceptibility; Jerseys and Shorthorns compared.

Thus from the experimental animals here one is not able to gather any sound evidence of the belief that pregnancy is associated with an increased capacity for resistance to tuberculosis. On the other hand, there is no evidence to the contrary.

Susceptibility of organs undergoing a sudden accession of functional activity during pregnancy.

While one is not able to arrive at any conclusion based on our own experiments as to the influence of pregnancy on the *general* susceptibility to tuberculosis, one can nevertheless point out some rather striking instances which show that those organs which then undergo a sudden increase in functional activity are remarkably liable to become affected during that period.

Susceptibility of the Mammary Gland.

The evidence is stronger in the case of the uterus than the mammary gland. Let us take the latter first. I have already mentioned that in Heifers 233 and 247 simultaneously injected with the same material tuberculosis was minimal, and differed in the two animals in that it had affected the mammary gland in the one which was pregnant when inoculated, but not in the other. But since the former lived four months after parturition it is possible that the mammary gland became affected during lactation. Another instance of tuberculosis spreading to the mammary gland in a pregnant heifer occurred in No. 251, in which likewise the tuberculosis elsewhere was minimal. In three other pregnant heifers, Nos. 235, 245, and 259, subcutaneously injected, the tuberculosis did not reach the mamma. Nor did it reach the uninjected quarters in the pregnant cow, No. 295, which had been injected in the other quarters of the udder. In two of the former the disease elsewhere was severe and steadily progressing. In the others it was minimal.

Tuberculosis of the mamma occurred in a non-pregnant heifer, No. 239, which received a subcutaneous injection of a virus of Group 1. It developed very little tuberculosis elsewhere, but the udder became distinctly enlarged, and yielded a secretion as though it were about to become functionally active, and in this secretion tubercle bacilli were present. It was killed six months after injection, and at the post-mortem examination the gland was found hypertrophied, but there were no tubercles seen in it. Secretion taken from the milk ducts after death caused tuberculosis in guinea pigs which were injected with it.

Tuberculosis of the mammary gland does not commonly occur in calves, or in heifers which have not become pregnant, even among those which develop very acute general tuberculosis. In addition to the above-mentioned animal, it has indeed only occurred here four times, namely, in Heifer 249, and in Calves 477, 575, and 597, all injected subcutaneously. Seven other non-pregnant heifers which received a subcutaneous injection of a virus of Group 1 developed general tuberculosis, but the mammary gland remained normal.

To sum up—among nine heifers which were not pregnant when injected with a virulent virus, tuberculosis of the mammary gland occurred in two, while among five pregnant heifers it occurred twice. In one cow which was pregnant, which received an intramammary injection, tuberculosis did not spread to the non-inoculated quarters of the udder. Therefore the evidence that the mammary gland becomes more susceptible to tuberculosis during pregnancy is not very strong. The evidence that the uterus does so is far clearer.

The following table contains the tests made upon guinea pigs with the milk of [1] the five heifers which calved during an experimental infection produced by a subcutaneous inoculation; and [2] the uninoculated quarters of the udder of Cow 295, which calved after an intramammary injection of the other quarters; and includes also the test made upon guinea pigs with the mammary secretion of Heifer 239, which was not pregnant. The milk of Heifer 249 was not tested.

GUINEA-PIGS INJECTED WITH MILK OF HEIFERS PREGNANT WHEN INFECTED WITH TUBERCULOSIS.

Virus.	Number of bovine.	Interval between testing of milk and birth of calf.	Quarter of udder from which milk was taken.	Number of guinea-pig.	Killed or Died.	Duration of life in days.		Result.
						Intrap.	Subcut.	
H 14. "F.S."	Heifer 235	19 days after	L.F.	70	K.	79	—	No tuberculosis.
			"	71	"	—	79	"
			L.H.	72	"	79	—	"
			"	73	"	—	79	"
			R.F.	74	"	79	—	"
			"	75	"	—	48	"
			R.H.	76	"	79	—	"
			"	77	"	—	48	"
H 19. "S.W."	Heifer 233	14 days after	L.F.	93	K.	77	—	No tuberculosis.
			"	94	"	—	77	"
			L.H.	95	"	77	—	"
			"	96	"	—	58	"
			R.F.	97	"	77	—	"
			"	98	"	—	77	"
			R.H.	99	"	58	—	Tuberculous.
			"	100	"	—	34	"
		54 days after	L.F.	206	K.	61	—	No tuberculosis.
			"	207	"	—	61	"
			L.H.	208	"	61	—	Tuberculous.
			"	209	"	—	61	No tuberculosis.
			R.F.	210	"	61	—	"
			"	211	"	—	61	"
			R.H.	212	"	61	—	Tuberculous.
			"	213	"	—	28	"
		125 days after	L.F.	314	K.	95	—	No tuberculosis.
			"	315	"	—	95	"
			L.H.	316	"	95	—	Tuberculous.
			"	317	"	—	95	"
			R.F.	318	"	95	—	No tuberculosis.
			"	319	"	—	95	"

GUINEA-PIGS INJECTED WITH MILK OF HEIFERS PREGNANT WHEN INFECTED WITH TUBERCULOSIS—*cont.*

Virus.	Number of bovine.	Interval between testing of milk and birth of calf.	Quarter of udder from which milk was taken.	Number of guinea-pig.	Killed or Died.	Duration of life in days.		Result.
						Intrap.	Subcut.	
H 16. "**J.H.**"	Heifer 245 -	6 days after -	L.F.	452	K.	64	—	No tuberculosis.
			"	453	"	—	64	"
			L.H.	454	"	64	—	"
			"	455	"	—	64	"
			R.F.	456	"	64	—	"
			"	457	"	—	64	"
			R.H.	458	"	64	—	"
			"	459	"	—	64	"
H 29. "**M.F.**"	Heifer 251 -	3 days after -	L.F.	463	K.	55	—	No tuberculosis.
			"	464	"	—	55	"
			L.H.	465	D.	3/24	—	Cause of death unknown.
			"	466	K.	—	55	Tuberculous.
			R.F.	467	D.	54	—	"
			"	468	K.	—	55	"
			R.H.	469	D.	54	—	"
			"	470	K.	—	55	"
H 10. "**B.S.**"	Heifer 259 -	2 days after -	L.F.	661	D.	95	—	No tuberculosis.
			"	662	K.	97	—	"
			L.H.	663	"	97	—	"
			"	664	"	97	—	"
			R.F.	665	"	97	—	"
			"	666	"	97	—	"
			R.H.	667	"	97	—	"
			"	668	"	97	—	"
		3 days after -	L.F.	669	K.	96	—	No tuberculosis.
			"	670	"	96	—	"
			L.H.	671	"	96	—	"
			"	672	"	96	—	"
			R.F.	673	"	96	—	"
			"	674	"	96	—	"
			R.H.	675	"	96	—	"
			"	676	"	96	—	"
H 10. "**B.S.**"	Cow 295 - - Non-inoculated quarters only.	95 days before -	L.H.	38	K.	51	—	No tuberculosis.
			"	39	"	—	51	"
			R.F.	40	"	51	—	"
			"	41	"	—	51	"
		1 day before -	L.H.	274	K.	52	—	No tuberculosis.
			"	275	"	—	52	"
			R.F.	276	"	52	—	"
			"	277	"	—	52	"

GUINEA-PIGS INJECTED WITH SECRETION OF THE UDDER OF A HEIFER NOT PREGNANT WHEN INFECTED WITH TUBERCULOSIS.

Virus.	Number of bovine.	Interval between testing of milk and birth of calf.	Quarter of udder from which milk was taken.	Number of guinea-pig.	Killed or Died.	Duration of life in days.		Result.
						Intrap.	Subcut.	
H 19. "**S.W.**"	Heifer 239 -	Not pregnant - Secretion obtained from udder after death.	L.F.	116	D.	7	—	No tuberculosis.
			L.H.	117	"	18	—	Tuberculous.
			R.F.	118	K.	19	—	"
			R.H.	119	D.	51	—	"

THE INFLUENCE OF LACTATION ON THE SUSCEPTIBILITY OF THE MAMMARY GLAND.

There is no complete evidence bearing on the susceptibility of the mammary gland to tuberculosis during *lactation*, owing to the absence of control injections of cows with resting udders.

But the very rapidly fatal tuberculosis which followed the injection of the milk ducts in some of our cows both here and at Walpole might be thought to be in favour of a great susceptibility of this organ when functionally active. On the other hand, when very small doses were used, as in Cow 74 at Walpole, and Cow 295 at Blythwood, the tuberculosis which followed was slow and in the latter case limited; so that on the whole the evidence on this point is inconclusive.

SUSCEPTIBILITY OF THE PREGNANT UTERUS.

The non-pregnant uterus has never been found affected with tuberculosis, no matter how severe the disease was elsewhere. The uterus has invariably been found healthy in heifer calves, and was so in the nine yearling heifers injected with viruses of Group I, which did not become pregnant. On the other hand, three out of six heifers which were pregnant when injected developed tuberculosis of the uterus, and gave birth to tuberculous calves.† This is a remarkably high proportion, and is the more striking because in none of the three cases was the tuberculosis very acute, and in one, while the mammary gland was also affected, there was very little tuberculosis elsewhere.

Two of these heifers, No. 235 and 245, had slowly but steadily progressing severe tuberculosis of the perlsucht type, affecting chiefly the lungs, when killed 176 and 209 days respectively after subcutaneous injection. Another [No. 251] was in good condition when killed 138 days after subcutaneous injection, and nine days after calving. She was found to have a caseous nodule in the mammary gland, and active tuberculosis in the cotyledons of the uterus. The tuberculosis elsewhere was minimal.

Detailed information concerning these animals is given in the following table.

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† See Report on congenital tuberculosis at Blythwood Farm, page 261.

TABLE SHOWING THE INFLUENCE OF PREGNANCY UPON TUBERCULOSIS OF UTERUS AND MAMMARY GLAND, GIVING ALL THE HEIFERS WHICH CALVED DURING AN EXPERIMENTAL INFECTION.

No. of mother.	Mode of inoculation.	Virus.	Interval between inoculation of mother and birth of calf.	Interval between birth of calf and death of mother.	Condition of great organs.	Condition of mamma.	Result of milk injections into guinea-pigs.	Condition of uterus.	Result of post-mortem on calf.
Shorthorn heifer 235	S.	H.14. "F.S."	153 days	23 days	Very advanced Perlsucht disease of lungs and pleura. Few tubercles in kidneys and one suprarenal. Minute ulcers in gut, and caseous tubercles in mesenteric glands.	Normal	All four quarters tested 19 days after birth of calf. No tuberculosis.	Early caseation in the cotyledons of the uterine mucous membrane.	Caseous patches and foci in hepatic glands. Emulsions of these glands, of liver, and of a pneumonic lobule in the lung, caused tuberculosis in guinea-pigs.
" " 233	S.	H.19. "S.W."	55 days	126 days	Very slight tuberculosis. Several caseous tubercles in thoracic glands and spleen. Three tubercular bodies on lung, loosely attached.	Induration of right hind quarters, in which tubercles are sparsely scattered.	All four quarters tested on several occasions. Guinea-pigs injected from both hind quarters became tubercular, those from fore quarters remained healthy.	Normal	Normal throughout.
Jersey heifer 245 -	S.	H.16. "J.H."	193 days	16 days	Extensive Perlsucht disease of lungs and pleura. Abdominal organs but slightly affected.	Normal	All four quarters tested 6 days after birth of calf. No tuberculosis.	Uterus appeared normal, but tubercle bacilli were found in a cotyledon.	Few foci in hepatic gland, in which tubercle bacilli were found.
" " 251 -	S.	H.29. "M.F."	129 days	9 days	Minimal tuberculosis. Small local lesion; calcareous foci in prescapular and thoracic glands. One tubercle in heart.	A caseous mass in left fore quarter. Other quarters appeared normal.	All four quarters tested 3 days after birth of calf. The milk of all the quarters except the left fore infected the guinea-pigs.	The cotyledons were large. From one, when cut, purulent material oozed out. This contained T.B. Besides this, numerous yellow foci were seen in the mucous membrane, in which T.B. were found.	Numerous early caseating foci in hepatic glands. Few early tubercles in lungs, liver, spleen, kidney, thoracic and lumbar glands.
" " 259 -	S.	H.10. "B.S."	409 days	4 days	Minimal tuberculosis. Calcareous nodule at seat of inoculation, one in prescapular gland. Two doubtful tubercles in left bronchial gland.	Normal	All four quarters tested on two occasions. Guineapigs remained healthy.	Normal	Normal throughout.
" cow 295 - -	M.	H.10. "B.S."	178 days	1 day (died)	Small caseous tubercles, and a few larger nodules in the inoculated quarters of the mamma. A few pinhead foci in supra-mammary gland. No tuberculosis elsewhere.	Non - inoculated quarters normal.	All four quarters tested on several occasions. The milk from inoculated quarters only caused tuberculosis.	Normal	Normal throughout.

When one considers these three instances of tuberculosis of the pregnant uterus, giving an incidence of no less than 50 per cent. among the pregnant animals, and contrasts it with the total absence of tuberculosis of that organ among the heifers which were not in calf when injected, many of which were much more severely and acutely affected than these three, one cannot, I think, escape the conclusion that pregnancy greatly increases the susceptibility of the uterus to tuberculosis.

Variation in the capacity for resistance of differential organs.

Before leaving this part of the subject it may be interesting to mention that, quite apart from pregnancy or lactation, the relative susceptibility of the different organs often appeared different in different animals. Thus of the animals which succumbed to acute tuberculosis some died of respiratory trouble; and in them the brunt of the disease was found to have fallen upon the lungs. Others died without dyspnœa being a marked symptom; and in these the lungs still contained a large amount of functional tissue, but the liver and spleen were more affected than in the animals first mentioned. A good example of this difference is afforded by Calves 967 and 969, H10. B.S., which both received 10 milligrammes of the same culture, and both of which had reached a dying condition on the same day, the one with very severe disease of the lungs, the other with plenty of functional lung tissue left, but with more severe disease of other organs than its fellow. Many other instances might be mentioned.

(c) *The Influence of Intercurrent Disease on Susceptibility to Tuberculosis.*

During the course of our work there have been many animals infected with tuberculosis, and some of them have suffered also from other diseases and intercurrent disorders. It is, of course, commonly believed that some diseases, as for example influenza, or even some slight departure from the normal state of health, diminishes the power of resisting tuberculosis. It is therefore of interest to inquire whether these animals infected with intercurrent disease showed any diminished power of resisting tuberculosis. This inquiry is rendered possible because in almost every case of experimental infection of the calf two animals were given identical doses. If then one suffered from an intercurrent disorder, it is possible to compare the course of the tuberculosis in it with that in its fellow. One must, of course, be on one's guard against differences which are due to differences of inherent individual susceptibility, by which I mean differences which are independent of any temporary departure from health. This subject will be dealt with in the next section.

Among the cases of intercurrent disease or disorder which affected one of two animals which received identical injections were many of more or less protracted diarrhœa, sometimes severe enough to cause a marked check to growth, and in one case [Calf 769. H45. F. M.] even to cause death. There were also many cases of more or less extensive carnification of the anterior parts of the lung, caused presumably by an old bronchial obstruction and consequent collapse. In one case there was death from pneumonia [Calf 619. H12. H. N.]. In another the ureters were blocked by gravel and there was acute hydronephrosis of both kidneys. In yet another [Calf 351. H12. H. N.] gravel caused several attacks of renal colic, the nature of which were not at first understood, and ultimately led to blocking of the urethra and extravasation of urine. In one calf an intravenous injection of an emulsion of a softened tubercular tumour from another calf caused both tuberculosis and pyœmia.

Only one of these animals [Calf 877. H28. C. L.] was injected with a virus of the human Group I., which is virulent for bovines. This was the calf which had hydronephrosis from blocking of the ureters. It died a little sooner than its fellow, having general tuberculosis, but death was thought to be due directly to the state of the urinary apparatus.

The other calves, which have been mentioned as having suffered from some intercurrent disease, were all injected with viruses of the human Group II. Even the intercurrent disease did not enable the latter to cause a progressive tuberculosis, nor did it have, as a rule, any obvious effect on the course of the tuberculosis. In Calf 769, which died of diarrhœa 17 days after subcutaneous injection of the virus H45. F. M., tubercle bacilli, it is true, were found widely distributed, but subsequent investigation showed that this was no exception to the general rule. (See the report on the distribution of non-virulent bacilli in the calf. p. 219.) In Calf 955, which had received a subcutaneous injection of 100 milligrammes of culture of Virus H.56. " F. T.", there was found rather extensive carnification of the anterior lobes of both lungs, and atrophy of a considerable portion of one kidney, associated with a deposit of triple phosphate in the corresponding calyces. The animal was never well, and scarcely gained in weight during three months, and yet the tubercular disease had not extended beyond the prescapular lymphatic gland, and was no more severe than in the fellow calf, which had remained well, and grown normally. In Calf 619, which died of pneumonia 83 days after subcutaneous injection of 50 mg. of culture, many little calcareous foci were found, but as these were retrogressive lesions and the pneumonia was recent, they could not be ascribed to its influence, and moreover it seems only reasonable to regard this as one of the more susceptible animals, among which such lesions are not uncommonly produced by viruses of the human Group II. Calf 351 is an interesting instance, because the dose had been enormous (3.140,000,000 bacilli), and in spite of this and the chronic disease of the kidney, the tuberculosis had made no progress beyond the glands nearest the seat of injection. In 409, which had received an intravenous injection, it is possible that the pyœmia may have assisted the tubercle bacilli to multiply in the body, for they were certainly very numerous everywhere—more numerous, I think, than one usually finds after an intravenous injection of a virus of the human Group II.—and had extended to the pyæmic joints.

Many of the rabbits suffered from severe coccidiosis of liver, which sometimes proved fatal; others from an extreme degree of infection with cysticerci, which not infrequently was found to have caused more or less chronic peritonitis and perihepatitis. Neither of these conditions so weakened the resistance of the animal as to enable viruses of the human Group II. to cause a progressive infection, nor did it appear that they had any influence on the severity of an infection produced, either by viruses of this group, or of the virulent Group I.

On the whole it may be said that there is but little evidence to be derived from our experiments here, in favour of the view that the course of tuberculosis is greatly influenced by intercurrent disease, or temporary disorders. On the contrary, it would appear that the resistance to invasion by tubercle bacilli is of a special kind, and not dependent to any great extent upon the *general* state of health of the body.

The evidence of this is, however, far from conclusive. The cases of intercurrent disease in which the virulent viruses of Group I. have been used are too few in number. Really trustworthy negative evidence could only have been obtained from animals injected with virulent viruses in minimal doses. And of such injections there have not been many. And none of them have presented cases of intercurrent disease.

Is a Diseased Part less able to resist Tuberculosis than a Corresponding Healthy Part of the same Animal?

As soon as it became known that after a subcutaneous injection of a virus of the human Group II. into the calf bacilli are distributed all over the body, and that they slowly disappear, it was obvious that one might put to the test of experiment the question whether the tubercle bacilli are more readily destroyed in a healthy than in a diseased part. The most convenient means of doing this was afforded by the frequent instances of carnification of lung. One might inject guinea pigs with approximately equal quantities of emulsion of carnified and normal lung from a calf which had previously been injected subcutaneously with one of the viruses of the human Group II.

This was done in two instances, namely, from Calf 955 and Calf 941. In the former case both the emulsions caused tuberculosis in the guinea pig, that made from the apparently normal part of the organ causing death first. In the latter case the emulsion of apparently normal lung caused tuberculosis, while that made from from the carnified lung killed the guinea-pigs which received it with acute peritonitis ten days later, without any tubercle bacilli being found in the exudation.

Thus the carnified lung does not appear to be more susceptible to tuberculosis than normal lung; but it must be admitted that up to the present the evidence on this point is of little or no value.

VARIATION IN CAPACITY FOR RESISTING TUBERCULOSIS IN NORMAL INDIVIDUAL ANIMALS.

Having dealt with the differences in the capacity for resisting tuberculosis, if any, which are due to differences of age, sex, and condition of general health, as well as with the differences of resistance exhibited by different organs in varying states of functional activity and of health, we now pass on to consider those differences which appear in healthy individuals of the same age, differences which cannot be attributed to any disturbance of general health, nor to any defect in any particular part or organ, which cannot be explained, but which are known as differences in the individual capacity for resistance. For, as we recognise that different species of animals differ in resistance capacity, so also do different individuals, though not to the same extent.

Differences of resisting power of a more or less marked kind among individuals have come to light from time to time among the large numbers of calves and other animals which have been injected in pairs, each individual of a given pair receiving exactly the same dose as the other, and being of the same age and weight. In such cases if there is no intercurrent disease, and the conditions under which the animals are kept are identical, one must attribute any differences in the course and severity of the disease to differences of individual resistance capacity.

I do not propose to dwell on the numerous instances where viruses of the human Group II. were used; for since these any incapable of setting up a progressive tuberculosis in any calf, they do not serve to bring out individual differences of resistance capacity in these animals strongly. Concerning these it is sufficient to recall the fact that many calves developed a few or more little translucent non-progressive tubercles in their lungs or other organs, while their fellows did not, and that the presence of these little bodies was thought to indicate a lower degree of resistance power than the average.

EVIDENCE OF VARIATION IN THE CAPACITY OF RESISTANCE TO TUBERCULOSIS AMONG CALVES AFFORDED BY ANIMALS INJECTED WITH *TISSUE EMULSIONS* OF THE HUMAN GROUP I.

Passing on then to the virulent viruses of Group I., we may consider first the evidence afforded by calves injected with *tissue emulsions.*

The following table contains all the instances of two animals receiving precisely similar injections of this kind. The only possible comparison is between the two individuals of the same pair, because different pairs received not only different viruses, but different doses also. All the pairs of animals are given, whether they show any difference in resistance capacity or no, in order that the judgment may be formed of the relative frequency and importance of these departures from the normal power of resistance. In order to facilitate the study of this table, animals which differ markedly in resistance capacity are printed in italics.

TABLE CONTAINING ALL THE PAIRS OF CALVES, EACH MEMBER OF WHICH RECEIVED AN IDENTICAL DOSE OF A VIRULENT EMULSION OF A TUBERCULOUS TISSUE.

SHOWING THE EVIDENCE OF VARIATION IN THE CAPACITY FOR RESISTING TUBERCULOSIS OF DIFFERENT INDIVIDUAL CALVES.

Virus.	Dose in estimated numbers of bacilli.	No. of calf.	Weight in kilos.	Duration of experiment, and condition at death.	Result.	No. of calf.	Weight in kilos.	Duration of experiment, and condition at death.	Result.
H. 29, "M.F." - -	3,020,400,000	479	58·05	Killed when dying 32 days	G.T. 3	477	83·0	Killed when dying - 34 days	G.T. 3
H. 28, "C.L." - -	901,466,000	513	65·75	Killed when very ill - 25 „	G.T. 2	515	50·80	„ „ „ - 44 „	G.T. 3
H. 32, "Y.W." - -	654,160,000	537	79·38	Killed when dying - 33 „	G.T. 3	533	62·13	„ „ „ - 35 „	G.T. 3
H. 14, "F.S." - -	362,000,000	125	48·50	„ „ „ - 23 „	G.T. 3	121	46·70	Killed when very ill - 36 „	G.T. 3
H. 17, Sp. "B." - -	150,520,000	555	74·85	Died - - - - 58 „	G.T. 3	531	71·21	Killed when dying - - 59 „	G.T. 3
H. 31, "L.F." - -	5,979,000	485	78·02	Killed when well - - 151 „	G.T. 1	491	71·21	Killed when well - - 152 „	G.T. 1
H. 16, "J.H." - -	*3,063,000*	*355*	*48·95*	*Killed when dying* - - *43* „	*G.T. 2*	*337*	*48·05*	„ „ „ - - *80* „	*G.T. 1 or 2*
H. 20, "F.L." - -	*2,301,000*	*213*	*92·96*	*Killed when well* - - *169* „	*G.T. 1*	*223*	*88·44*	„ „ „ - - *169* „	*L.T.*
„ „ - -	*2,301,000*	*517*	*76·67*	*Showed some illness* - *172* „	*G.T. 2*	*507*	*55·79*	„ „ „ - - *172* „	*S.T. or G.T. 1*
H. 19, "S.W." -	*1,546,000*	*159*	*60·77*	*Killed when ill* - - *50* „	*G.T. 3*	*179*	*62·13*	„ „ „ - - *114* „	*S.T.*
H. 2, Sp. "A." - -	*301,000*	*83*	*73·9*	*Killed when dying* - - *134* „	*G.T. 2*	*91*	*82·6*	„ „ „ - *147* „	*G.T. 1*
H. 7, "C.M." - -	*250,000*	*141*	*71·21*	„ „ „ - - *53* „	*G.T. 3*	*139*	*63·04*	*Killed when ill* - - *85* „	*G.T. 2*
„ „ - -	*48,000*	*105*	*34·9*	*Killed when ill* - - *47* „	*G.T. 3*	*103*	*41·27*	*Killed when well* - - *130* „	*G.T. 1*
„ „ - -	12,000	115	56·69	Killed when well - - 96 „	G.T. 1	117	63·50	Killed when well - - 97 „	G.T. 1
H. 10, "B.S." - -	*5,000*	*113*	*81·64*	*Killed when ill* - - *120* „	*G.T. 2*	*35*	*128·81*	*Killed when well* - - *106* „	*S.T.*
H. 2, Sp. "A." - -	*Unknown*	*153*	*62·58*	„ „ „ - - *39* „	*G.T.* 3	*163*	*53·52*	„ „ „ - - *64* „	*S.T.*

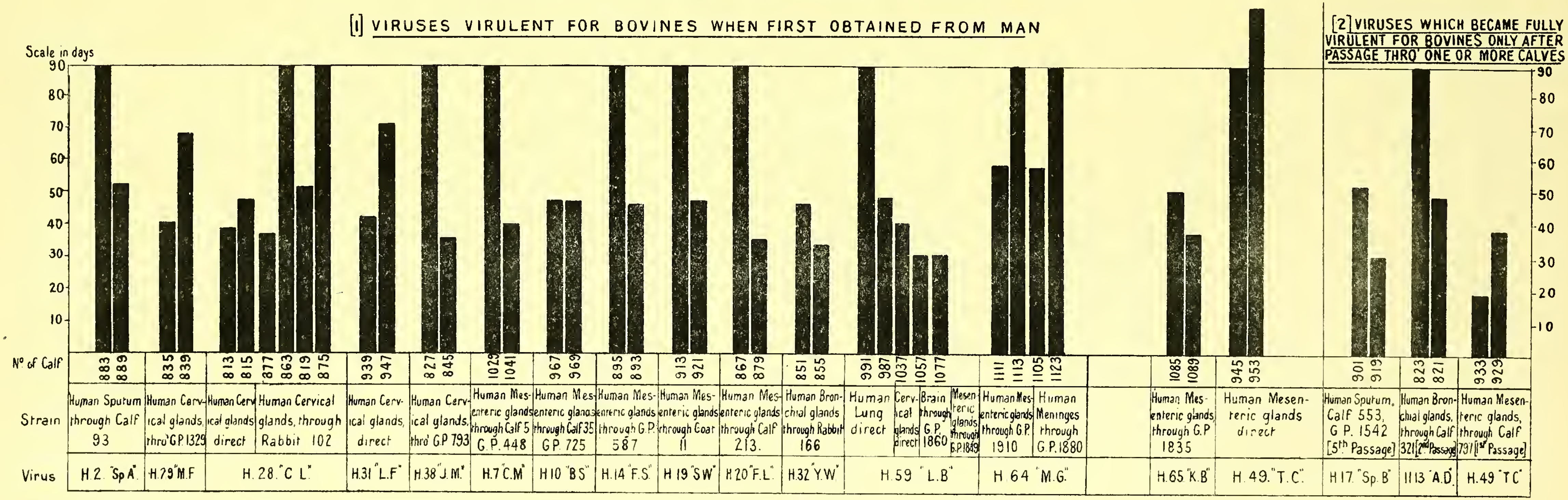
NOTE.—(*a*) The details of each pair of injections into calves which received identical doses, and which may therefore be compared with one another, are placed in a single line across the page. The italics are to draw attention to the instances where the difference in the result was greatest

(*b*) In this table.
G.T. 1. = General Tuberculosis, slight.
G.T. 2. = „ „ moderate.
G.T. 3. = „ „ severe.

STRAINS OF
HUMAN ORIGIN

CHART SHOWING THE LENGTH OF LIFE IN DAYS OF CALVES AFTER SUBCUTANEOUS INJECTION OF 10 MILLIGRAMMES OF CULTURE

[ALL CALVES WHICH REACHED THE 90 DAY LIMIT WERE KILLED WHEN IN GOOD HEALTH, WITH THE EXCEPTION
OF Nº 953, (H.49."T.C") WHICH DIED AFTER 108 DAYS
AND OF Nº 1123 (H64 "M.G") WHICH WAS IN POOR HEALTH WHEN KILLED]



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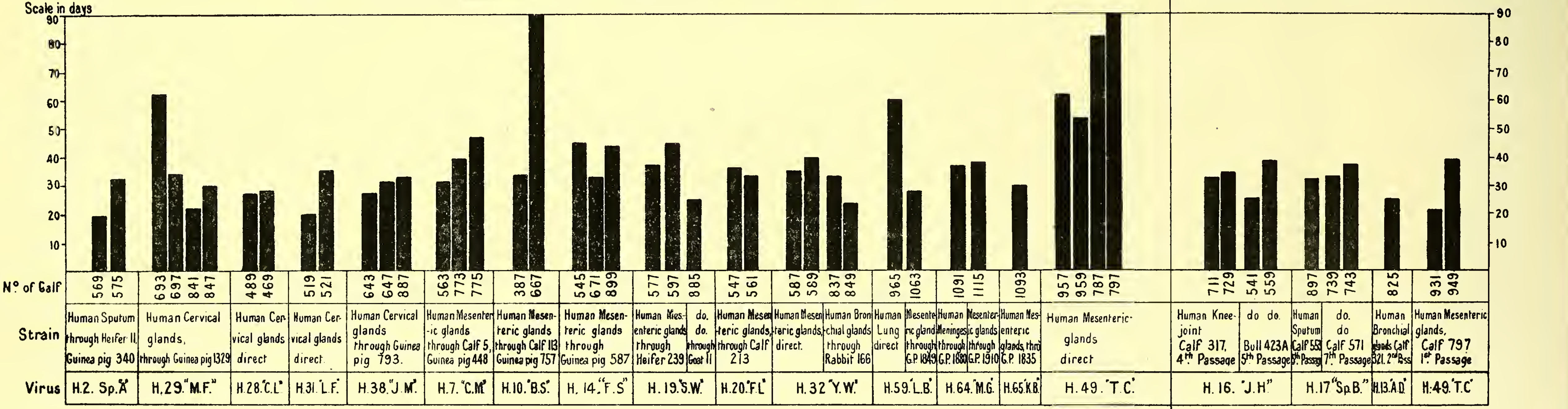
HUMAN ORIGIN.

CHART SHOWING THE LENGTH OF LIFE IN DAYS OF CALVES AFTER SUBCUTANEOUS INJECTION OF 50 MILLIGRAMMES OF CULTURE †

[A] VIRUSES VIRULENT FOR BOVINES WHEN FIRST OBTAINED FROM MAN.

[B] VIRUSES WHICH BECAME FULLY VIRULENT FOR BOVINES ONLY AFTER PASSAGE THROUGH ONE OR MORE CALVES *



† *Calf 512 received 30 milligrammes only, Calf 469, 43 milligrammes only, and Calf 1091, 45 milligrammes only, and Calf 887, 23 milligrammes only.*

Calf 667, which reached the 90 day limit, was in good health when killed, and was found to have general tuberculosis of moderate severity

Calf 797 also was killed when in good health and was found to have slight tuberculosis only. All the others died or were in a dying condition when killed & were found to have severe general tuberculosis.

* *H.49 "T.C.", when obtained from man (see calves 957 to 797) was slightly less virulent for Calves than other virulent viruses*

The other viruses of this group were originally incapable of causing a progressive tuberculosis in calves.

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The most marked instances of difference in resistance capacity occurred in Calves 153 and 163 [H2, Sp. A]. Calves 159 and 179 [H19, S. W.] and 213 and 223 [H20, F. L.]. The severity of the disease also differed in Calves 35 and 113 [H10, B. S.], but this seemed attributable to difference in age or size.

Nos. 153 and 163 received each 30 c.c. of an emulsion of the tuberculous organs of a guinea-pig. The former was killed when very ill 39 days later, and was found to have severe general tuberculosis. The latter remained well, and when killed 64 days after injection was found to have no lesions beyond the thoracic glands. In the second case Nos. 159 and 179 received each a dose of 10 c.c. of an emulsion of human mesenteric gland not very rich in tubercle bacilli. No. 159 was killed when ill, 50 days after injection, and was found to have severe general tuberculosis. The other was quite well when killed four months after injection, and had only, besides a scar at the seat of injection and a calcareous prescapular gland, a few minute foci scattered in the thoracic, hepatic, and mesenteric glands. In the last mentioned case Calves 213 and 223 each received 10 c.c. of an emulsion poor in bacilli. The former had a moderate amount of disease in the lungs and thoracic glands when killed; the latter only a minimal local lesion and a few calcareous grains in the nearest glands. It must be remembered that these instances represent extreme differences. In them the doses were relatively small. With large doses the results, as a rule, in two calves which received the same dose were very fairly uniform.

EVIDENCE OF VARIATION IN THE CAPACITY OF RESISTANCE TO TUBERCULOSIS AMONG CALVES AFFORDED BY ANIMALS INJECTED WITH *Cultures* OF THE HUMAN GROUP I.

The animals which were injected with culture fall into two groups, namely, those which received 50 mg. and those which received 10 mg. of bacilli subcutaneously. The majority of these animals died of tuberculosis or were killed when they were in a dying condition, the duration of life may therefore be taken as a rough relative measure of the capacity for resistance. In the few cases where the animals were killed when in more or less good general condition three months after injection, this period may be taken as the measure of maximum resistance capacity.

A strict comparison, of course, is only possible between the members of a group all of which received the same dose of the same culture, but since the differences of virulence among the viruses of this group, if they existed, were not marked enough to reveal themselves in our experiments,* H49. T. C. alone excepted, a rough comparison is possible between all the calves which received the same doses.

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ture.

The evidence as to the existence of variation in capacity for resistance to tuberculosis among these calves is best brought out by the diagrams which appear on p.   of this appendix, and in which the duration of life of each calf is shown by a column of proportionate height. If this be consulted it will be seen, among the calves injected with 50 mg. (those injected with H49 T. C. excepted), the duration of life was remarkably uniform, one calf alone living out the period of 90 days*, while all the rest were dying of tuberculosis on an average period of 33·5 days; the shortest being 20 and the longest 63 days.

If one compares together the duration of life of the two individuals of each pair one finds a remarkably close agreement, the widest discrepancy occurring with H10. B. S., where one animal, already mentioned, was killed when in good condition, after three months, and the other when dying on the 34th day.

We may conclude, therefore, that 50 mg. of a culture of the human Group I., when subcutaneously injected, almost invariably produces rapidly fatal tuberculosis in the calf, and is too large a dose to allow individual differences of resistance power to become manifest.

Evidence obtained from 10 mg. injections.

This is not the case when 10 mg. is the dose. Differences of individual resistance capacity are then often great enough to turn the scale from death to recovery. Thus of 41 calves injected with this dose of virulent culture* 30 died, or were killed when dying, in an average period of 44½ days, while 11 were in more or less good condition when killed three months after injection, and in some of these latter the lesions were only of very slight or of moderate extent.

Comparing together the duration of life of the two individuals forming each pair, one finds that in eight of the pairs both animals died, or were killed when dying; while in eleven one animal died of general tuberculosis, while the other lived out 90 days, as already stated, and was not very severely affected.

Thus in our experiments with 10 mg. doses the highly resistant individuals were remarkably uniformly distributed among the calves injected with different viruses. This was very fortunate; for it is obvious that if the resistant animals had come together, the results might have been very misleading.

From these considerations it is evident: *That there are considerable differences in individual powers of resistance among different calves;*

That these come more into evidence with 10 *milligramme than with* 50 *milligramme doses;*

That the differences of individual resisting power among calves are such that 10 *milligramme injections cannot be trusted to reveal small differences of virulence among different tubercular viruses.*

(2) THE INFLUENCE OF DOSE.

The influence of the number of tubercle bacilli injected, on the severity of the resulting disease, became apparent at the commencement of our work, and a series of guinea pigs injected by me at Walpole showed that the duration of life after injection bore a very close relation to the quantity of tuberculous material injected, whether this was introduced into the subcutaneous tissue or into the peritoneum. It was not, however, until the results of injecting bovine animals began to accumulate that the full effect of variations of dose became apparent, and it was evident that a tubercular virus which was capable of killing with certainty bovine animals within a few weeks when injected in large doses, would cause only mininal lesions, limited and non-progressive in these animals, if only a small dose were given. It will be sufficient to quote a few instances.

In the case of the culture H. 10 "B.S.," Heifer 249 and Calf 387 were subcutaneously inoculated each with 50 milligrammes of culture, while Heifer 259 and Calf 385 received of the same culture minimal qualities calculated to contain 1.800,000 tubercle bacilli. The two first-mentioned animals developed an acute general tuberculosis, which was rapidly approaching a fatal termination when they were killed 54 and 34 days respectively after injection; the other two, which received the small doses, remained well, and when killed 413 and 202 days later respectively, were found to have only small calcareous nodules at the seat of injection, and a few minute calcareous foci elsewhere.†

The difference of dose was very large in this instance, in the following it was smaller. An emulsion of the prescapular gland of Calf 355 (virus H. 16. "J.H.") was injected subcutaneously in various doses into four calves. The numbers of tubercle bacilli estimated in the qualities of emulsion injected were as follows: Calf 281, 10,900,000; calf 317, 3,000,000; Calf 343, 712,000; and Calf 371, 10,000. In the first three animals acute general tuberculosis resulted, and a fatal termination was approaching when the animals were killed, No. 281 54 days after the injection, and Nos. 317 and 343 in 80 and 68 days respectively. The one which received the smallest dose remained well, and only a few small non-progressive calcareous foci were found in various organs in addition to a calcareous local lesion and prescapular gland when the animal was killed 66 days after injection.

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* The actual culture injected into this animal was probably at fault, for rabbits also injected with it lived much beyond the usual period. That the strain was not attenuated was shown by the injection of calves and rabbits at a later period. These died in the usual time.

* H49 T. C. again excepted.

† For a full description of the post-mortem examinations and other details of the experiments mentioned here, *see* Vol. II. of the Appendix.

In the case of H. 13, "A.D.," three calves were subcutaneously injected with varying quantities of the same emulsion of tubercular tissue from the prescapular gland of Calf 301. The doses were 500 million, 3½ million, and 15 thousand tubercle bacilli respectively. No. 325, which received the largest dose, was killed when dying of general tuberculosis 24 days after injection. No. 321 was killed when very ill 63 days after receiving 3½ million, and was found to have general tuberculosis, while No, 315 which received only 15 thousand, remained well, and when killed 70 days later was found to have no more than a small calcareous lesion at the seat of injection, and very few minute and non-progressive foci elsewhere.

These examples will be sufficient to prove that minute doses do not produce progressive tuberculosis in calves and heifers, even when large doses of the same cause rapidly fatal tuberculosis.

We now pass on to the evidence of the influence of dose which is given by the culture injections. In these the differences were much smaller than in those already mentioned, yet they will be seen nevertheless to have had a marked influence on the result.

In the diagrams already referred to when writing of susceptibility, there are forty-eight calves injected with 50 milligrammes, one with 43, and one with 30 milligrammes, of virulent culture, and forty-three injected with 10 milligrammes of the same. If we exclude the strain H49. "T.C." derived directly from human mesenteric glands, as being probably of slightly lesser virulence than the others, there is, as already stated, no evidence that they differed from each other in virulence, at least they did not do so to an extent which became apparent in the course of these experiments.

In estimating the influence of dose, we may therefore compare the calves injected with 10 milligrammes as a whole with those injected with the larger quantity. The larger dose was fatal to all the forty animals, except one which survived 90 days, while eleven out of the forty-one animals injected with the smaller dose lived three months, and were then in very fair condition. Moreover, with few exceptions, those which died after an injection of 10 milligrammes lived longer than those which received 50 milligrammes of the same culture. The average duration of life of those which succumbed to 50 milligrammes was 33·7 days, while that of those which succumbed to 10 milligrammes was 44·5 days. These differences in the results of injecting 10 and 50 mg. are very clearly shown in the diagrams.

Differences in individual resisting power, however, sometimes had a more powerful result on the severity of the disease than even this difference of dose; and in half the cases where a pair of 10 milligramme calves may be compared with a pair of 50 milligramme calves, the more susceptible of the two former has died in about the same time as the less susceptible of the latter. In one instance the more susceptible 10 milligramme calf died before either of the 50 milligramme animals, while the remaining 10 milligramme calf died at the same time as the remaining 50 milligramme animal.

In rabbits, too, the number of tubercle bacilli injected influences the severity of the disease produced. Rabbits have been injected intraperitoneally with different doses of cultures of all our virulent viruses. These have usually included, 10, 1, 0·1, and 0·01 milligrammes, and the resulting disease, though always fatal, has been the more prolonged, the smaller the dose. This rule has not been without exceptions, differences in individual susceptibility in these animals, as in the calves, not unfrequently overcoming the influence of numbers of bacilli. For the details of the evidence upon which this statement rests, the table of culture injections into rabbits may be consulted.

In one important respect the influence of dose is different in the rabbit and the bovine race. In the latter, injected with a virus of the human Group I., the disease may cease to spread and the animal recover, if the dose be small enough. In the former it appears that there is no dose so small that it will not produce a progressive tuberculosis.

Differences of Pathogenicity between Cultures of Tubercle Bacilli and Emulsions of Tuberculous Tissue.

It will be quite clear from the instances given above that the number of tubercle bacilli injected have a marked influence on the course of the resulting disease when these bacilli happen to be virulent for the class of animal to which the recipient belongs. But there are other factors included in the term dose besides the numbers of tubercle bacilli and their comparative virulence.

In our experience, in order to cause severe tuberculosis with virulent cultures, it is necessary to inject far larger numbers of bacilli than when emulsions of tuberculous tissue are used. In the chapter on the stability of virulence of tubercle bacilli in artificial culture it is estimated that that bacilli injected along with the tuberculous tissue in which they have developed are at least as effective as 100 times as many from an artificial culture of three weeks' growth.

REPORT ON CONGENITAL TUBERCULOSIS AT BLYTHWOOD FARM.

Dr. L. COBBETT.

Many guinea-pigs and rabbits born of tubercular mothers have been killed and examined here, and no case of tuberculosis has been seen by me among them.

Six calves have been born of mothers artificially infected with viruses of the human Group I, and of these three were born tuberculous.

The mothers of those which were healthy at birth had only a very small amount of tuberculosis. Two received very small doses. No. 295 was injected with a quantity of tissue emulsion estimated to contain one million tubercle bacilli into each of two quarters of the udder. She gave birth to a calf 179 days later, and died soon after. The tuberculosis was minimal and limited to the infected quarters of the mamma and to the supra-mammary lymphatic gland. The calf was free from tubercle but died two days after birth. No. 259 received a subcutaneous injection of culture estimated to contain approximately two million bacilli. She calved nearly fourteen months after injection when well and fat, and was killed four days later, and found to have minimal lesions only.

The third normal calf was born of Heifer 233 two months after a subcutaneous injection of a tissue emulsion estimated to contain 53 million tubercle bacilli. The mother was not killed until four months later; besides a small local lesion, she had one or two minute tubercles in the lungs and spleen, and scattered tubercles in one quarter of the mammary gland which was indurated.

The remaining three heifers which calved during an experimental infection, gave birth to tuberculous calves, and each suffered from disease of the uterus. In two there was extensive advanced tuberculosis of lungs of the Perlsucht type. No. 235 received a subcutaneous injection of emulsion containing 4½ million tubercle bacilli: she gave birth to a calf five months later, and was killed three weeks after parturition. The calf reacted well with tuberculin. It was killed twenty-six days after birth. It was found to have caseous patches in the portal lymphatic glands, and a suspicious focus in the lungs. An emulsion of a portion of lung and also one of a portion of the liver which appeared healthy gave rise to tuberculosis after injection into guinea-pigs.

No. 245 received a subcutaneous injection estimated to contain 157 million tubercle bacilli. She gave birth to a calf six months later and was killed sixteen days after parturition. There was extensive disease of the lungs, and but little tuberculosis elsewhere, but a few caseating foci were found in the cotyledons of the uterus. The calf was killed when five days old. A few tubercles were found in its portal lymphatic glands.

The third heifer, No. 251, is even more interesting, because it developed tuberculosis of the uterus and mammary gland, and gave birth to a tuberculous calf, when there was no tuberculosis elsewhere, except at the seat of injection, and a few little retrogressive foci in the nearest prescapular, and in the thoracic glands. The mother received a subcutaneous injection of a tissue emulsion estimated to contain 2,800 million tubercle bacilli; she remained apparently well, and gave birth to a calf four months afterwards. Nine days after parturition she was killed. There was extensive tuberculosis of the cotyledons of the uterus, and a caseous mass in the left fore-quarter of the mammary gland. The calf was killed six days after birth. In its portal lymphatic gland numerous early caseating foci were found; and there were also a few early tubercles in the lungs, liver, spleen, and kidneys, and in the thoracic and lumbar glands.*

The proportion of calves born of tuberculous mothers and affected with congenital tuberculosis is undoubtedly far larger than that which is believed to occur under natural conditions. The numbers are small—three out of six—yet too large to be altogether exceptional. How, then, can this exceptionally large incidence be explained? An experimental tuberculosis is no doubt often a far more acute and rapidly fatal form of the disease than that which usually occurs under natural conditions. Yet this does not explain the high incidence of congenital tuberculosis here, for the mothers in no cases were suffering from the acute form of the disease, and in one, the disease, except in the uterus, was very slight. It will be observed that the proportion of uterine disease was very high, and that congenital tuberculosis did not occur without it. I am inclined to attribute the frequency of uterine disease to the conjunction of two causes: (1) The susceptibility during pregnancy of that organ, as well as of others which undergo a great increase of functional activity, and (2) the distribution of bacilli in large numbers by the blood stream which follows on subcutaneous injection. The last condition probably does not occur under natural conditions, and is, I believe, the dominating reason why congenital tuberculosis has been comparatively so common in the course of our experimental investigations.

LOUIS COBBETT.

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* A summary of the particulars of these animals will be found on page 253.

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